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Direct Comparison of Cardiac Myosin-Binding Protein C With Cardiac Troponins for the Early Diagnosis of Acute Myocardial Infarction

BACKGROUND: Cardiac myosin-binding protein C (cMyC) is a cardiac-restricted protein that is more abundant than cardiac troponins (cTn) and is released more rapidly after acute myocardial infarction (AMI). We evaluated cMyC as an adjunct or alternative to cTn in the early diagnosis of AMI.

METHODS: Unselected patients (N=1954) presenting to the emergency department with symptoms suggestive of AMI, concentrations of cMyC, and high-sensitivity (hs) and standard-sensitivity cTn were measured at presentation. The final diagnosis of AMI was independently adjudicated using all available clinical and biochemical information without knowledge of cMyC. The prognostic end point was long-term mortality.

RESULTS: Final diagnosis was AMI in 340 patients (17%). Concentrations of cMyC at presentation were significantly higher in those with versus without AMI (median, 237 ng/L versus 13 ng/L, $P<0.001$). Discriminatory power for AMI, as quantified by the area under the receiver-operating characteristic curve (AUC), was comparable for cMyC (AUC, 0.924), hs-cTnT (AUC, 0.927), and hs-cTnI (AUC, 0.922) and superior to cTnI measured by a contemporary sensitivity assay (AUC, 0.909). The combination of cMyC with hs-cTnT or standard-sensitivity cTnI (but not hs-cTnI) led to an increase in AUC to 0.931 ($P<0.0001$) and 0.926 ($P=0.003$), respectively. Use of cMyC more accurately classified patients with a single blood test into rule-out or rule-in categories: Net Reclassification Improvement +0.149 versus hs-cTnT, +0.235 versus hs-cTnI ($P<0.001$). In early presenters (chest pain <3 h), the improvement in rule-in/rule-out classification with cMyC was larger compared with hs-cTnT (Net Reclassification Improvement +0.256) and hs-cTnI (Net Reclassification Improvement +0.308; both $P<0.001$). Comparing the C statistics, cMyC was superior to hs-cTnI and standard sensitivity cTnI ($P<0.05$ for both) and similar to hs-cTnT at predicting death at 3 years.

CONCLUSIONS: cMyC at presentation provides discriminatory power comparable to hs-cTnT and hs-cTnI in the diagnosis of AMI and may perform favorably in patients presenting early after symptom onset.

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Clinical Perspective

What Is New?

- Cardiac myosin-binding protein C is a recently described novel biomarker of cardiac injury, and in small proof-of-concept studies its serum concentration rises and falls more rapidly than that of troponin T and I.
- This is the first study to assess the diagnostic and prognostic value of cardiac myosin-binding protein C in patients presenting with possible acute myocardial infarction.
- A rule-in/rule-out pathway using the novel biomarker was designed to compare discriminative power in a clinical setting.

What Are the Clinical Implications?

- Diagnostic accuracy of cardiac myosin-binding protein C for acute myocardial infarction was similar to that of high-sensitivity cardiac troponin T and high-sensitivity cardiac troponin I in the entire cohort but superior for those with chest pain of <3 hours (early presenters) when compared with high-sensitivity cardiac troponin T.
- Cardiac myosin-binding protein C has correctly triaged more patients to rule-out or rule-in groups than either high-sensitivity cardiac troponin I or high-sensitivity cardiac troponin T, leaving a much smaller proportion in the observation groups. This advantage may facilitate early discharge of low-risk patients.

Of the 130 million attendances to emergency departments (EDs) in the United States each year, ≈7 million (6%) are a result of acute chest pain.[1] The assessment and triage of such patients has become increasingly complex because now only a small proportion of those with acute myocardial infarction (AMI) have the diagnostic ECG change of ST-segment elevation.[2] Consequently, the identification of patients with AMI has become almost totally dependent on the measurement in the systemic circulation of cardiac troponin (cTn) I or cTnT. These biomarkers are released slowly.[3] To overcome this hurdle, the analytic performance of the cTn assays has been enhanced markedly to measure the lower concentrations achieved before the late peak.[4] Hence, the best assays can reliably measure cTn concentrations below the 99th percentile of the healthy population. These high-sensitivity (hs) assays are increasingly available and are the subject of national and international guidelines describing their use to achieve more rapid triage.[5,6] In particular, the European guidelines recommend the use of assays for hs-cTnI and hs-cTnT to rapidly rule in and rule out AMI. Algorithms using widely spaced decision limits based on concentrations well below the population-defined 99th percentile (for rule-out) and above the 99th percentile (for rule-in) markedly improve the sensitivity of rule-out and specificity of rule-in. However, many patients presenting with chest pain have cTn concentrations that place them between these decision limits, in an indeterminate observation zone. These patients require repeat testing and subsequent second or third rounds of triage based on rates of change of cTn concentration over time.[6–8] European guidelines also do not support the use of rapid rule-out/rule-in pathways using hs-cTn in patients presenting too early after chest pain onset—only after 3 hours is the rule-out threshold at the limit of detection guideline-compliant.[6] This introduces systemic delays in allocation of evidence-based treatments and prolongs stay in the pressured and precious environment of the ED.

Originally discovered by Offer et al[9] in 1973, the myosin-binding protein C family consists of 3 isoforms specific for slow skeletal, fast skeletal, and cardiac muscle, the latter being exclusively expressed in the heart from neonatal throughout human development.[10,11] Among others,[12–15] we have identified cardiac myosin-binding protein C (cMyC; Figure 1) as a new candidate biomarker of cardiac injury.[16] In common with cTnT and cTnI, cMyC expression is restricted to the heart but is more abundant.[17] Moreover, cMyC rises more rapidly in the systemic circulation than hs-cTnT after timed, iatrogenic AMI,[16] perhaps as a result of its higher myocardial concentration.[18] Using a recently developed hs assay for cMyC,[19] a pilot study in 26 patients presenting early with AMI suggested that cMyC may rise more rapidly than hs-cTnI.[20]

The purpose of the current study is to compare the novel biomarker cMyC (measured on a research platform) against the most accurate currently available biochemical signals, hs-cTnI and hs-cTnT, for the early detection of AMI.

METHODS

Study Design and Population

The APACE study (Advantageous Predictors of Acute Coronary Syndrome Evaluation) is an ongoing international multicenter diagnostic study (9 study centers in Switzerland, Spain, Poland, the Czech Republic, and Italy) designed to advance the early diagnosis of AMI.[4,21–23] All patients >18 years of age presenting to the ED with acute chest discomfort possibly indicating AMI were eligible for recruitment if the onset of or peak chest pain symptoms were within the preceding 12 hours. Enrollment was independent of renal function, whereas patients with terminal kidney failure on chronic dialysis were excluded. For this analysis, the following patients were excluded (Figure I in the online-only Data Supplement): patients presenting with ST-segment elevation myocardial infarction, patients with missing levels of cMyC at presentation, and patients in whom the final diagnosis

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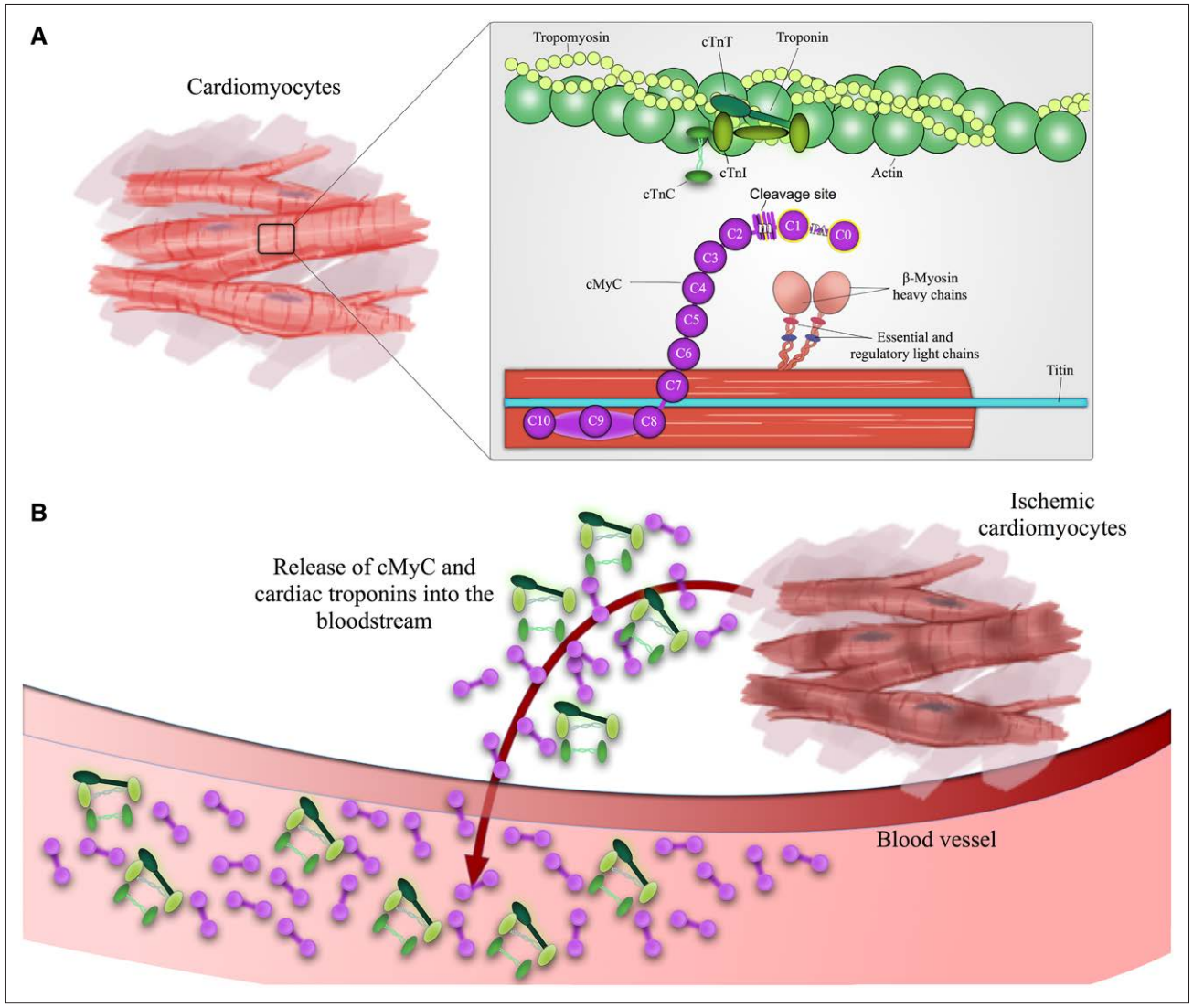

Figure 1. Depiction of cardiac troponin and cardiac myosin-binding protein C release during myocardial injury. Structure of cardiac myosin-binding protein C and cardiac troponins in (**A**) healthy cardiomyocytes and (**B**) ischemia-induced cardiomyocyte damage. The highlighted N-terminal domain C0C1 is the binding site for the previously developed monoclonal antibodies used for detection of the cardiac-specific isoform of cMyC.[16] cMyC indicates cardiac myosin-binding protein C; cTnI, cardiac troponin I; and cTnT, cardiac troponin T.

remained unclear after adjudication and at least 1 hs-cTnT level was elevated. This group is comprised of patients triaged and discharged after a negative gold-standard test at the time of enrollment (on a conventional cTn assay), who were later found to have an elevated hs-cTn result (Table I in the online-only Data Supplement). A proportion of patients had no levels of cMyC measured at presentation because of insufficient sample volume. Demographics of the patients excluded because of missing cMyC values, compared with those of the test cohort, appear in Table II in the online-only Data Supplement. The protocol for routine clinical assessment is also described in the online-only Data Supplement. To obtain follow-up data, patients were contacted 3, 12, 24, and 36 months after discharge via telephone, email, or letter. Additionally, information regarding death during follow-up was obtained from the patient's hospital notes, the family physician's records, and the national registry on mortality.

The study was carried out according to the principles of the Declaration of Helsinki and approved by the local ethics committees. Written informed consent was obtained from all patients. T.K., R.T., and C.M. had full access to all the data in the study and take responsibility for its integrity and the data analysis. The authors designed the study, gathered and analyzed the data according to the STARD guidelines (Standards for Reporting Diagnostic accuracy studies) for studies of diagnostic accuracy (Table III in the online-only Data Supplement), vouch for the data and analysis, wrote the paper, and decided to publish.

Adjudicated Final Diagnosis

Adjudication of the final diagnosis was performed centrally according to the universal definition of MI, incorporating

levels of hs-cTnT as the adjudicating biomarker.[24] It was based on extensive patient documentation derived from 2 sets of data. First, clinical data were derived from routine clinical investigations, including all available medical records (eg, patient history, physical examination, results of laboratory testing including serial local hs-cTn, radiological testing, ECG, echocardiography, cardiac exercise stress test, lesion severity and morphology at coronary angiography) pertaining to the patient from the time of ED presentation to 90-day follow-up. Second, study-specific assessment was collected, including 34 chest pain characteristics and serial hs-cTnT measurements to take advantage of the higher sensitivity and higher overall diagnostic accuracy offered by the more sensitive assays, as previously published.[4,21] In situations of disagreement about the diagnosis, cases were reviewed and adjudicated in conjunction with a third cardiologist. In brief, AMI was diagnosed when evidence indicated myocardial necrosis in association with a clinical setting consistent with myocardial ischemia. Myocardial necrosis was diagnosed by ≥1 (h)s-cTn value above the 99th percentile together with a significant rise or fall.[25–27] All other patients were classified into the categories of unstable angina, cardiac but noncoronary disease (eg, tachyarrhythmias, perimyocarditis), noncardiac chest pain, and symptoms of unknown origin.

Measurement of cMyC, hs-cTnI, hs-cTnT, and Standard-Sensitivity cTnI

Blood samples for the determination of cMyC, hs-cTnI, hs-cTnT, and standard-sensitivity (s) cTnI were collected into heparin plasma and serum tubes at presentation to the ED and serially thereafter (at time points 1 h, 2 h, 3 h, and 6 h). Serial sampling was discontinued when a diagnosis of AMI was certain and treatment required patient transfer to the coronary care unit or catheter laboratory. After centrifugation, samples were frozen at −80°C until they were assayed in a blinded fashion in a dedicated core laboratory. cMyC was measured using the previously established hs assay on the Erenna platform performed by Millipore Sigma.[19] The assay has a limit of detection of 0.4 ng/L and a lower limit of quantification of 1.2 ng/L. The 99th percentile cutoff point determined previously (in patients without obstructive coronary artery disease on invasive angiography) is 87 ng/L.[19] Details of the assays used for hs-cTnI, hs-cTnT, and s-cTnI are described in the online-only Data Supplement.

Early Guideline-Based Triage and Net Reclassification Improvement

The European Society of Cardiology (ESC) has published a rapid rule-in/rule-out pathway in the 2015 non-ST-segment elevation MI guidelines using hs-cTn at 0 hours and 1 hour to risk-stratify patients into rule-out, observe, and rule-in categories.[6] Such categorization did not drive clinical decisions in this cohort, but it was used to compare the potential clinical utilities of cMyC and hs-cTn as triage tools. For this purpose, we have compared the categorical discrimination of hs-cTnT, hs-cTnI, and cMyC at presentation only (without subsequent delta measurements). In brief, the ESC pathway classifies patients, based on the presentation sample at 0 hours, into rule-out with an hs-cTnT level <5 ng/L and hs-cTnI <2 ng/L and into rule-in (for both assays) at ≥52 ng/L.[6] The ESC advocates the use of the pathway only in patients with ≥3 hours since chest pain onset; for completeness, we have presented results for all patients, <3 and ≥3 hours since chest pain onset alone.

For cMyC we separated the cohort into derivation and validation cohorts (a randomized 3:7 split; for comparison see Table IV in the online-only Data Supplement). The rule-out threshold was derived from a predefined sensitivity of ≥99.5% and rule-in from a predefined specificity >95% for the gold-standard diagnosis of AMI. This resulted in a rule-out threshold of ≤10 ng/L and a rule-in threshold of >120 ng/L for cMyC (Figure II in the online-only Data Supplement). These thresholds were then used in the validation cohorts to compare cMyC against both hs-cTnT and hs-cTnI. Net Reclassification Improvement (NRI) operates as follows. Each patient is first assigned a classification (rule-out, observe, or rule-in) based on cutoff values of hs-cTnI/hs-cTnT in the presentation blood sample (the initial model). The same cohort is then reclassified to the same 3 groups based on the cMyC cutoff values (the new model). This reclassification may correctly or incorrectly reallocate a patient (eg, a patient who went on to be diagnosed with an AMI may be correctly reclassified from observe to rule-in or incorrectly reclassified from observe to rule-out). The NRI analysis defines separate categorical NRI values for those patients who were ultimately diagnosed with AMI (NRI_{AMI}) and those who were not (NRI_{noAMI}; (range, −1 to +1). Dimensionless NRI reflects the unweighted net movement of all patients regardless of final diagnosis (range, −2 to +2). NRI_{AMI} is positive if there is a net movement of patients with adjudicated AMI into higher risk classifications using cMyC (the new model). NRI_{noAMI} is positive when a net movement of patients occurs without an adjudicated diagnosis of AMI into lower risk classifications using cMyC (the new model).[28] NRI calculations were performed for the validation cohort, early presenters (<3 hours since onset of chest pain; ESC guideline not applicable), and late presenters (≥3 hours since onset; ESC guideline applicable). Tables are presented in full where appropriate.

Statistical Analysis

All data are expressed as medians (first quartile, third quartile) or means (SD) for continuous variables (compared with the Mann-Whitney *U* test or Student *t* test) and for categorical variables as numbers and percentages (compared with Pearson χ^2). Hypothesis testing was 2-tailed, and *P* values <0.05 were considered statistically significant. No adjustment for multiple comparisons was performed.

Discrimination power was quantified by the area under the receiver-operating characteristics curve (AUC) for each biomarker with all cases available, using 1000 stratified bootstrap replicates to calculate confidence intervals (CIs). Logistic regression was used to combine cMyC levels with hs-cTnT, hs-cTnI, or s-cTnI values for the assessment of an incremental value using 2 biomarkers at presentation. Subgroup analysis was performed for patients presenting early, defined as chest pain onset ≤3 hours of presentation to the ED. This is a particular limitation of the published ESC guidance on the use of hs-cTn for risk stratification because the rapid rule-out/

rule-in algorithms are only applicable to patients with chest pain onset >3 hours.

Predictive value of the biomarkers during follow-up was assessed 2-fold: We calculated (1) Harrell's C statistic for each biomarker at presentation for end points AMI, death or the composite of AMI, and all-cause mortality during follow-up (excluding the index event), and a higher C index indicates a higher probability of an event occurring during follow-up with higher biomarker values[29]; and (2) Kaplan-Meier survival curves. Cox regression analysis was performed as follows. All available biomarker levels were divided into quintiles and groups according to rule-out, observe, and rule-in classification. Unadjusted Cox proportional hazard regression models were fitted for 30-day and 3-year follow-up for each group with the lowest quintile (or risk group, respectively) normalized to a hazard ratio of 1 and assessed using the likelihood-ratio test. Cox coefficients and thus hazard ratios were not calculated if the lowest risk group did not suffer any events, which would invalidate the regression model. NRI statistics were calculated as categorical values.[28,30] The integrated discrimination improvement values quoted reflect a category-free (positive or negative) change in model performance. CIs for cutoff thresholds, NRI, and integrated discrimination improvement statistics were derived using 1000 bootstrap replicates. All statistical analyses were performed using R, version 3.3.0 GUI 1.68 (The R Foundation for Statistical Computing), including packages ggplot2, R Markdown, RStudio, PredictAbel, survival, Hmisc, compare, and ROCR.

RESULTS

Baseline Characteristics

A total of 1954 unselected patients eligible for this analysis were enrolled (Figure I in the online-only Data Supplement). Median age was 62 years, 31% were women, and 36% had a prior history of coronary artery disease (Table 1). Overall, 1469 patients (75%) had no significant electrocardiographic abnormalities at presentation to the ED. Median time since onset of chest pain was 5 hours (interquartile range [IQR], 3, 12), with a median of 3 hours (IQR 2, 7) since peak chest pain severity.

The adjudicated final diagnosis was AMI in 340 (17%) patients, unstable angina in 10%, symptoms of cardiac origin other than coronary artery disease in 14%, noncardiac symptoms in 54%, and symptoms of unknown origin in 5%.

Median follow-up for the entire cohort was 772 days (IQR 731, 907); of those not sustaining any events in the monitoring period (AMI or death), the median follow-up was 792 days (IQR 738, 923). A total of 165 (8%) patients died during the 3-year follow-up; 1903 patients (97%) exceeded 90 days of follow-up; of those who did not (n=51, 3%), 27 (1%) sustained a cardiovascular death.

Distribution of Biomarker Concentrations

As shown in Figure 2, cMyC levels were significantly higher in patients with AMI (n=340) compared with patients with other diagnoses (AMI, median 237 ng/L [IQR 71, 876 ng/L]; unstable angina, median 21 ng/L [IQR 13, 43 ng/L]; cardiac symptoms of origin other than coronary artery disease, median 33 ng/L [IQR 12, 96 ng/L]; noncardiac symptoms, median 10 ng/L [IQR 6, 19 ng/L]; symptoms of unknown origin, median 11 ng/L [IQR 7, 16 ng/L]; $P<0.001$ for all comparisons with patients with AMI). Similarly, blood concentrations of hs-cTnT, hs-cTnI, and s-cTnI were significantly higher in AMI compared with other final diagnoses (median biomarker concentrations are displayed in Tables V and VI in the online-only Data Supplement). Overall, blood concentrations of cMyC in relation to the limit of detection were higher than those of hs-cTn in all diagnostic categories (Table V in the online-only Data Supplement). Noncardiac sources of cMyC variation were previously investigated in an ambulatory cohort.[19] Results of comparison within the groups with AMI and noncardiac symptoms have been displayed in Tables VII and VIII in the online-only Data Supplement.

Discrimination Power

In blood drawn at presentation, the discrimination of cMyC for AMI, as quantified by the AUC, was 0.924 (95% CI, 0.910–0.939), compared to the AUCs for hs-cTnT 0.927 (95% CI, 0.913–0.941; $P=0.573$ for direct comparison), hs-cTnI 0.922 (95% CI, 0.908–0.936; $P=0.993$ for direct comparison), and s-cTnI 0.909 (95% CI, 0.889–0.928; $P=0.024$ for direct comparison) (Table 2, Figure 3).

Early Presenters

In patients presenting ≤3 hours of symptom onset (n=694, with AMI adjudicated in 16%), the AUC for cMyC was 0.915 (95% CI, 0.887–0.941), compared with the AUCs for hs-cTnT, 0.892 (95% CI, 0.857–0.922; $P=0.022$); hs-cTnI, 0.909 (95% CI, 0.879–0.935; $P=0.539$); and s-cTnI, 0.892 (95% CI, 0.859–0.925; $P=0.060$) (Table 2).

Combination of cMyC With cTn

AUC for the combination of cMyC with hs-cTnT was 0.935 (95% CI, 0.921–0.948; $P=0.002$ for comparison with hs-cTnT alone); cMyC with hs-cTnI, 0.929 (95% CI, 0.913–0.943; $P=0.093$ for comparison with hs-cTnI alone), and cMyC with s-cTnI, 0.928 (95% CI, 0.909–0.943; $P<0.001$ for comparison with s-cTnI alone) (Table 2, Figure III in the online-only Data Supplement).

Table 1. Demographics

Demographics	All Patients (N=1954)	AMI (n=340)	Other Diagnoses (n=1614)	*P* Value*
Age, y	62±16	69±13	60±16	<0.001
Male	1341 (69)	256 (75)	1085 (67)	0.004
Risk factors				
Hypertension	1247 (64)	269 (79)	978 (61)	<0.001
Hyperlipidemia	992 (51)	227 (67)	765 (47)	<0.001
Diabetes mellitus	369 (19)	92 (27)	256 (16)	<0.001
Current smoking	500 (25)	90 (27)	386 (24)	0.345
History of smoking	718 (38)	141 (42)	577 (36)	0.051
History				
Coronary artery disease	710 (36)	174 (51)	536 (33)	<0.001
Previous myocardial infarction	474 (24)	118 (35)	356 (22)	<0.001
Previous revascularization (CABG or PCI)	553 (28)	127 (37)	426 (26)	<0.001
Peripheral artery disease	119 (6)	43 (13)	76 (5)	<0.001
Previous stroke	100 (5)	23 (7)	77 (5)	0.167
Vital status				
Heart rate, beats/min	79±20	81±20	79±20	0.092
Systolic blood pressure, mm Hg	144±24	145±27	143±24	0.421
Diastolic blood pressure, mm Hg	82±15	81±17	82±15	0.299
Electrocardiographic findings				
ST-segment depression	193 (10)	93 (28)	100 (6)	<0.001
T-wave inversion	260 (13)	82 (24)	178 (11)	<0.001
No significant electrocardiographic abnormalities	1469 (75)	161 (49)	1308 (83)	<0.001
Laboratory assessment				
Estimated glomerular filtration rate, ml/min/1.73m^2*†	84±26	74±26	86±25	<0.001
Presentation time				
Time since chest pain onset, h	5 [3, 12]	5 [3, 12]	5 [3, 12]	0.898
Time since chest pain peak, h	3 [2, 7]	3 [2, 7]	4 [2, 7]	0.408

AMI indicates acute myocardial infarction; CABG, coronary artery bypass graft; and PCI, percutaneous coronary intervention.

**P* values for comparison AMI group versus all other diagnoses. Data are expressed as medians (first quartile, third quartile] or means±SD and for categorical variables as numbers (percentages).

†Glomerular filtration rate was estimated using the Modification of Diet in Renal Disease formula.

Classification Function of Cutoff Values for Risk Groups

Sensitivity, specificity, and negative and positive predictive values were calculated for derivation (Tables IX and X in the online-only Data Supplement) and validation cohorts based on cutoffs published in the 2015 ESC guideline.[6] In the validation cohort (n=1368,233 events), hs-cTnT has a sensitivity of 99.6% (95% CI, 98.5–100) and negative predictive value of 99.7% (95% CI, 99–100) at the rule-out threshold of 5 ng/L, and a specificity of 97.1% (95% CI, 96.1–98) and positive predictive value of 80.1% (95% CI, 73.2–86.2) at the rule-in threshold (52 ng/L); and hs-cTnI has a sensitivity of 100% (95% CI, 100–100) and negative predictive value of 100% (95% CI, 100–100) at 2 ng/L, and a specificity of 94.5% (95% CI, 93–95.8) and positive predictive value of 70.4% (95% CI, 63.6–76.5) for rule-in (Tables 3 and 4). After obtaining clinically meaningful cutoff thresholds in the internal derivation cohort (based on sensitivity ≥99.5% and specificity >95%; Tables IX and X and Figure II in the online-only Data Supplement), these were tested in the validation cohort. At a threshold of 10 ng/L for rule-out, cMyC achieves a sensitivity of 99.6% (95% CI, 98.6–100) and negative predictive value of 99.8% (95% CI, 99.3–100). At 120 ng/L for the rule-in threshold, cMyC achieves a specificity of 94.7% (95% CI, 93.3–95.9) and positive predictive value of 71% (95% CI, 64.9–77.2) (all data are listed in Tables 3 and 4).

All data for the groups of early (<3 hours of chest pain) and late presenters (≥3 hours of chest pain) are presented in Tables XI and XII in the online-only Data Supplement. In short, in early presenters, cMyC demonstrates higher sensitivity than hs-cTnT (100% versus 98.8%) and greater specificity (46.4% versus 33.3%) at

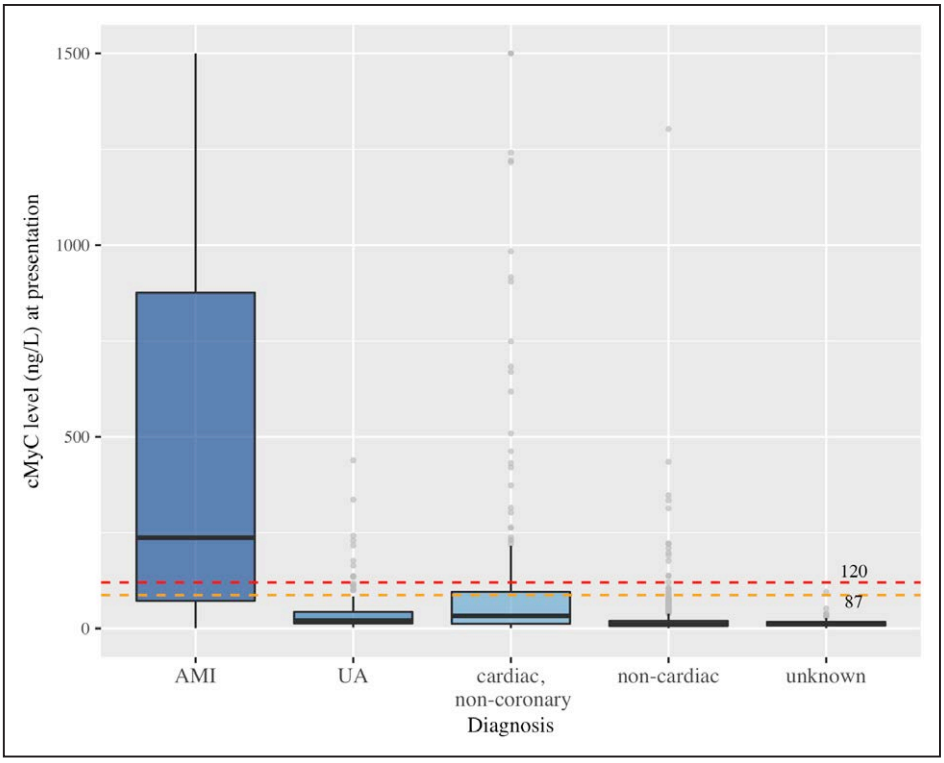

Figure 2. Baseline distribution of cMyC levels at presentation to the emergency department in all patients based on adjudicated final diagnosis.

Boxes represent interquartile ranges (IQR). Whiskers extend to 1.5*IQR from the hinges (*y* axis capped at 1500 ng/L, outliers represented by light gray bullets); 87 ng/L represents the 99th percentile based on a previous study and 120 ng/L the cutoff threshold for diagnostic rule-in of AMI at presentation. AMI, median, 237 ng/L (IQR 71, 876 ng/L); unstable angina, median, 21 ng/L (IQR 13, 43 ng/L); cardiac symptoms of origin other than coronary artery disease, median, 33 ng/L (IQR 12, 96 ng/L); noncardiac symptoms, median, 10 ng/L (IQR 6, 19 ng/L; symptoms of unknown origin, median, 11 ng/L (IQR 7, 16 ng/L) (*P*<0.001 for all comparisons with patients with AMI). AMI indicates acute myocardial infarction; cMyC, cardiac myosin-binding protein C; and UA, unstable angina.

the rule-out threshold (10 ng/L). Sensitivity is similar for cMyC and hs-cTnI, however, again with greater specificity for cMyC (47.1% versus 23.2%). In the group of late presenters, cMyC yields higher specificity (37.3% versus hs-cTnT, 28.4%; 38.1% versus hs-cTnI, 15.9%) at the rule-out threshold with otherwise comparable sensitivity. Specificity for adjudicated diagnosis of AMI was individually assessed at the 99th percentile in Table XIII in the online-only Data Supplement.

Risk Group Reclassification

The distribution of patients in risk groups rule-out, observe, and rule-in based on the initial blood test (either hs-cTnT, hs-cTnI, or cMyC) is displayed in Figure 4 (validation cohort, n=1368, AMI in 17%). cMyC classified 443 patients (32.4%) safely as rule-out, compared with 348 (25.4%) with hs-cTnT and 206 (15.1%) with hs-cTnI, predominantly by reducing the size of the observation group.

In the validation cohort (Tables 3 and 4), cMyC at presentation was superior to hs-cTnT with NRI +0.149 (NRI_{noAMI} +0.081, NRI_{AMI} +0.068; *P*<0.001) and to hs-cTnI with NRI +23.5 (NRI_{noAMI} +0.226, NRI_{AMI} +0.009; *P*<0.001). In the cohort of early presenters (<3 hours of chest pain), cMyC was superior to hs-cTnT with NRI +0.256 (NRI_{noAMI} +0.256, NRI_{AMI} +0.128; *P*<0.001) and to hs-cTnI with NRI +0.308 (NRI_{noAMI} +0.257, NRI_{AMI} +0.051; *P*<0.001) (Table XI in the online-only Data Supplement). In the cohort of late presenters (≥3 hours of chest pain), cMyC was superior to hs-cTnT with NRI +0.133 (NRI_{no-AMI} +0.084, NRI_{AMI} +0.049; *P*<0.001) and to hs-cTnI with NRI +0.227 (NRI_{noAMI} +0.240, NRI_{AMI} -0.012; *P*<0.001) (Table XII in the online-only Data Supplement).

Prognostic Performance of cMyC

As quantified by Harrell's C statistic calculated from the presentation sample (Table XIV in the online-only Data Supplement), cMyC matched the performance of hs-cTnT in predicting AMI (excluding index event), death, and the composite end point within a 3-year follow-up. Compared with hs-cTnI, there was a statistically different but numerically small improvement in predicting death and the composite end point at 3 years: cMyC C index 0.767 versus hs-cTnI 0.732 (*P*=0.001) and 0.746 versus 0.722 (*P*=0.008), respectively; AMI was comparable.

Table 2. Area Under the Receiver-Operating Characteristics Curve: Comparisons Between Biomarkers

Patient Groups	AUC (95% Confidence Interval)	*P* value	n
All patients: comparison			
cMyC vs. hs-cTnT	0.924 (0.910–0.939) vs. 0.927 (0.913–0.941)	0.573*	1554 controls, 322 AMI
cMyC vs. hs-cTnI	0.923 (0.908–0.937) vs. 0.922 (0.908–0.936)	0.993*	1537 controls, 320 AMI
cMyC vs. s-cTnI	0.924 (0.906–0.938) vs. 0.909 (0.889–0.928)	0.024*	1463 controls, 311 AMI
Early presenters (≤ 3 h since chest pain onset): comparison			
cMyC vs. hs-cTnT	0.915 (0.887–0.941) vs. 0.892 (0.857–0.922)	0.022*	562 controls, 104 AMI
cMyC vs. hs-cTnI	0.915 (0.889–0.939) vs. 0.909 (0.879–0.935)	0.539*	554 controls, 102 AMI
cMyC vs. s-cTnI	0.914 (0.888–0.939) vs. 0.892 (0.859–0.925)	0.060*	529 controls, 103 AMI
All patients: combination cMyC with…			
hs-cTnT	0.935 (0.921–0.948)	0.002†	1548 controls, 322 AMI
hs-cTnI	0.929 (0.913–0.943)	0.093†	1537 controls, 320 AMI
s-cTnI	0.928 (0.909–0.943)	<0.001†	1463 controls, 311 AMI

AMI indicates acute myocardial infarction; AUC, area under the curve; cMyC, cardiac myosin-binding protein C; hs-cTnI, high-sensitivity cardiac troponin I; hs-cTnT, high-sensitivity cardiac troponin T; and s-cTnI, standard-sensitivity cardiac troponin I.

*P value for direct comparison between biomarkers.

†P value for direct comparison between AUC for combination (cMyC with cTn) and respective cTn on its own.

cMyC was significantly better at predicting AMI, death, or the composite end point when compared with cTnI.

For the calculation of cumulative hazard ratios for all-cause mortality using a Cox regression model, each biomarker was separated into quintiles. The hazard ratios for hs-cTnT at 3-year follow-up were 2.3 (95% CI, 0.6–9.0) in the second quintile, 7.7 (95% CI, 2.3–25.8) in the third quintile, 17.7 (95% CI, 5.5–57.1) in the

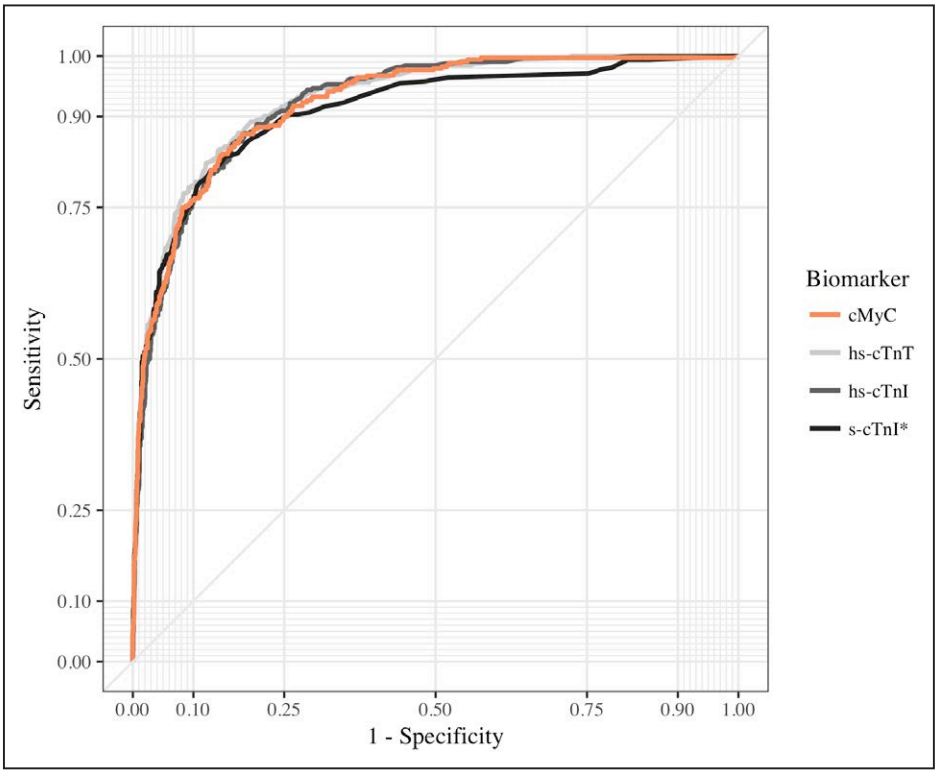

Figure 3. Receiver operating characteristic (ROC) curves for individual biomarkers.
Diagnostic performance of cMyC, hs-cTnT, hs-cTnI, and s-cTnI in the early diagnosis of acute myocardial infarction (AMI) based on presentation blood sample and adjudicated AMI diagnosis. ROC curves describing the performance of cMyC (orange line; area under the curve [AUC], 0.924), hs-cTnT (light gray line; AUC, 0.927), hs-cTnI (dark gray line; AUC, 0.922), and s-cTnI (black line; AUC, 0.909*) (*P<0.05). cMyC indicates cardiac myosin-binding protein C; hs-cTnI, high-sensitivity cardiac troponin I; hs-cTnT, high-sensitivity cardiac troponin T; and s-cTnI, standard-sensitivity cardiac troponin I.

Table 3. Net Reclassification Improvement: hs-cTnT (Validation Cohort)

Initial Model	New Model—cMyC (10/120)—Validation Cohort					
	No AMI (n=1089)			AMI (n=219)		
hs-cTnT	Rule-Out	Observe	Rule-In	Rule-Out	Observe	Rule-In
Rule-out	249	77	0	0	1	0
Observe	190	509	32	1	66	24
Rule-in	0	7	25	0	9	118
NRI	0.081 (95% CI, 0.029–0.113)			0.068 (95% CI, 0.016–0.121)		
NRI (dimensionless)	0.149 (95% CI, 0.089–0.210); *P* value <0.001			IDI		0.050 (95% CI, 0.029–0.070)
Thresholds						
hs-cTnT 5 ng/L	Sensitivity (95% CI): 99.6% (98.5–100)	NPV (95% CI): 99.7% (99–100)		Specificity (95% CI): 29.9% (27.3–32.5)		PPV (95% CI): 22.2% (19.6–24.8)
hs-cTnT 52 ng/L	Sensitivity (95% CI): 58.1% (51.6–64)	NPV (95% CI): 92% (90.5–93.5%)		Specificity (95% CI): 97.1% (96.1–98)		PPV (95% CI): 80.1% (73.2–86.2)
cMyC 10 ng/L	Sensitivity (95% CI): 99.5% (98.6–100)	NPV (95% CI): 99.8% (99.3–100)		Specificity (95% CI): 38.8% (35.8–41.7)		PPV (95% CI): 24.6% (21.8–27.4)
cMyC 120 ng/L	Sensitivity (95% CI): 64.9% (58.5–71.2)	NPV (95% CI): 93.1% (91.4–94.5)		Specificity (95% CI): 94.8% (93.5–96)		PPV (95% CI): 71.5% (64.7–78)

AMI indicates acute myocardial infarction, based on the adjudicated gold-standard diagnosis; CI, confidence interval; IDI, integrated discrimination improvement; NPV, negative predictive value; NRI, net reclassification improvement; and PPV, positive predictive value.

fourth quintile, and 33.6 (95% CI, 10.6–106.3) in the fifth quintile (*P*<0.05 for all except second quintile). The hazard ratios for hs-cTnI were 6.6 (95% CI, 1.5–29.2), 11.3 (95% CI, 2.7–48.3), 25.1 (95% CI, 6.1–103.3), and 39.7 (95% CI, 9.7–161.8), respectively (*P*<0.05 for all quintiles). The hazard ratios for cMyC were 2.6 (95% CI, 0.7–10.0), 7.8 (95% CI, 2.3–25.9), 17.2 (95% CI, 5.4–55.0), and 29.4 (95% CI, 9.3–93.2) (*P*<0.05 for all except second quintile). Survival curves for cMyC and hs-cTn assays are displayed in Figures IVA–C in the online-only Data Supplement for 3-year and 30-day follow-up.

DISCUSSION

To our knowledge, cMyC is the first cardiac-restricted protein to be analyzed as a diagnostic test for AMI since cTn. In this diagnostic multicenter study, we compared its diagnostic performance to cTnI and cTnT, measured using the leading hs assays recommended in current practice guidelines,[6] in a well-characterized and large cohort of patients presenting with symptoms suggestive of AMI. Discrimination for MI with cMyC was similar to that of hs-cTnT and hs-cTnI and superior to s-cTnI. In patients presenting <3 hours of chest pain onset,

Table 4. Net Reclassification Improvement: hs-cTnI (Validation Cohort)

Initial model	New model—cMyC (10/120)—Validation cohort					
	No AMI (n=1080)			AMI (n=224)		
hs-cTnI	Rule-out	Observe	Rule-in	Rule-out	Observe	Rule-in
Rule-out	167	32	0	0	0	0
Observe	273	526	22	1	63	19
Rule-in	0	25	35	0	16	125
NRI	0.226 (95% CI, 0.174–0.258)			0.009 (95% CI, −0.044–0.062)		
NRI (dimensionless)	0.235 (95% CI, 0.174–0.296); *P* value <0.001			IDI		0.078 (95% CI, 0.057–0.098)
Thresholds						
hs-cTnI 2 ng/L	Sensitivity (95% CI): 100% (100–100)	NPV (95% CI): 100% (100–100)		Specificity (95% CI): 18.4% (16–20.8)		PPV (95% CI): 20.3% (18–22.7)
hs-cTnI 52 ng/L	Sensitivity (95% CI): 62.9% (56.4–68.9)	NPV (95% CI): 92.5% (90.9–93.9)		Specificity (95% CI): 94.5% (93–95.8)		PPV (95% CI): 70.4% (63.6–76.5)
cMyC 10 ng/L	Sensitivity (95% CI): 99.6% (98.6–100)	NPV (95% CI): 99.8% (99.3–100)		Specificity (95% CI): 39.4% (36.3–42.4)		PPV (95% CI): 25.5% (22.9–28.5)
cMyC 120 ng/L	Sensitivity (95% CI): 64.3% (58.1–70.7)	NPV (95% CI): 92.8% (91.2–94.3)		Specificity (95% CI): 94.7% (93.2–96)		PPV (95% CI): 71.8% (65.3–77.9)

AMI indicates acute myocardial infarction, based on the adjudicated gold-standard diagnosis; CI, confidence interval; IDI, integrated discrimination improvement; NPV, negative predictive value; NRI, net reclassification improvement; and PPV, positive predictive value.

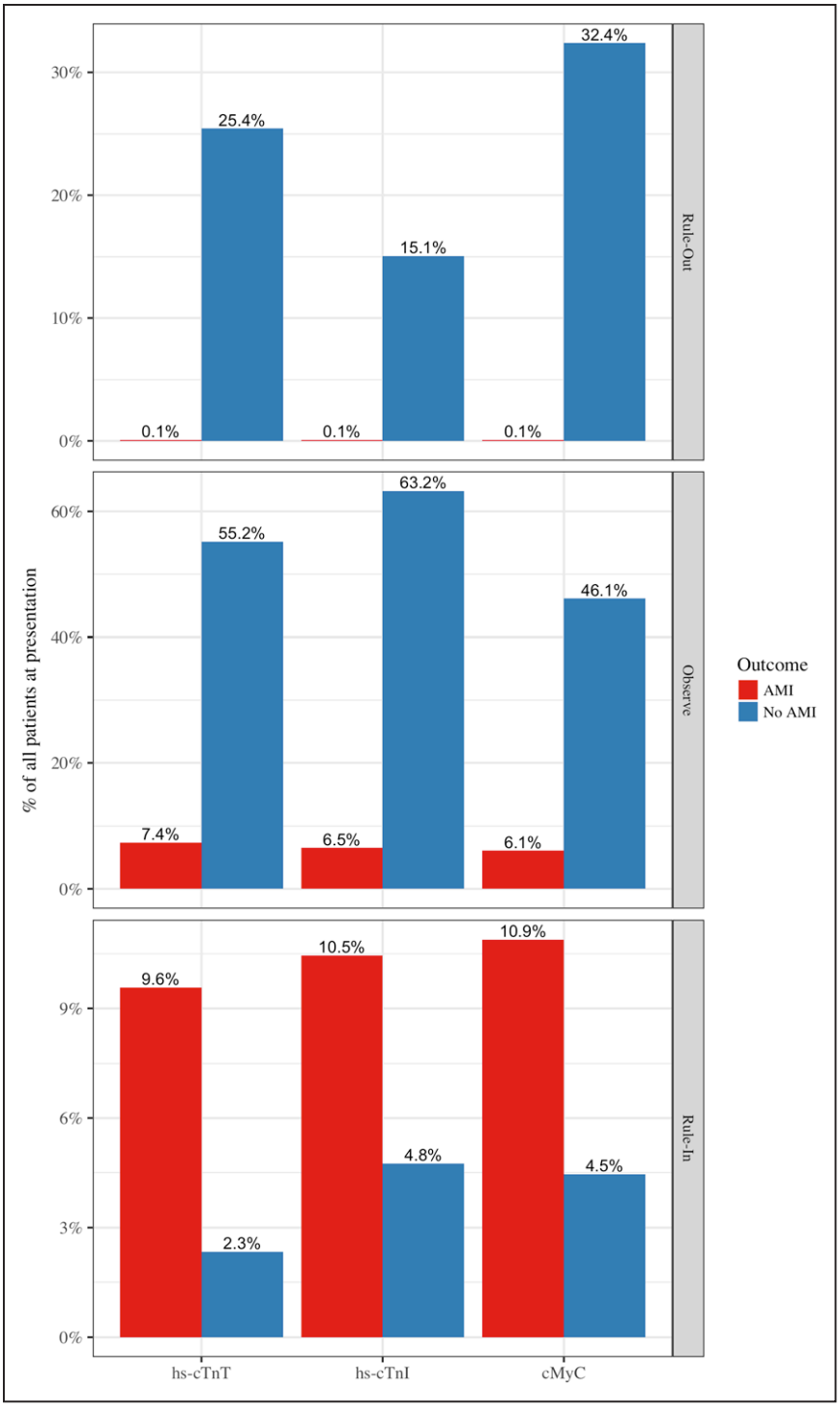

Figure 4. Distribution of patients in different risk categories after presentation blood tests. Data are based on European Society of Cardiology guideline 2015[6] for hs-cTnT and hs-cTnI and newly developed cutoff thresholds for cMyC at ≤10 ng/L for rule-out and >120 ng/L for rule-in of myocardial infarction. AMI indicates acute myocardial infarction; cMyC, cardiac myosin-binding protein C; hs-cTnI, high-sensitivity cardiac troponin I; and hs-cTnT, high-sensitivity cardiac troponin T.

cMyC was superior to hs-cTnT, despite the latter's use as the adjudicating biomarker. Using cutoffs for cMyC calibrated against those recommended in the guidelines,[6] cMyC correctly and safely rules out and rules in AMI in a greater proportion of patients than either hs-cTnT or hs-cTnI. These findings indicate that cMyC may be better able to triage patients presenting to the ED with suspected AMI.

cTnT and cTnI have transformed the management of patients with suspected AMI, and their importance is enshrined in the Universal Definition of Myocardial Infarction.[31] Consequently, AMI events are identified/adjudicated based on a significant rise or fall in cTnT/I blood concentration. This definition has harmonized clinical care and clinical research but also has introduced an inherent bias in favor of cTnT/cTnI versus novel diagnostic biomarkers in studies such as ours. cMyC is not part of the troponin complex and has a distinct location within the cardiac sarcomere (Figure 1). For these reasons, our findings regarding the performance of cMyC

against the hs-cTnT and hs-cTnI gold standard are notable. Because cMyC was not measured through the patients' journey, it is a matter of speculation whether the outcome would have been different with cMyC as the adjudicating biomarker.

After iatrogenic or spontaneous AMI, cMyC appears more rapidly in the systemic circulation than either hs-cTnT or hs-cTnI.[16,20] This finding is probably a result of a combination of cMyC's greater myocardial abundance, distinct sarcomeric location, and loose association with myosin and actin.[16] This biological distinctiveness of cMyC likely underpins the diagnostic advantage we observed over hs-cTnT/hs-cTnI in patients presenting <3 hours of symptom onset. Moreover, the more rapid appearance of cMyC in the systemic circulation after cardiac injury is also likely to explain the net reclassification improvement over both hs-cTnT and hs-cTnI.

No large prospective clinical trials compare the effect of different biomarkers of cardiac necrosis on clinical outcome. Nonetheless, it is interesting to speculate what effect the improved classification of events by cMyC could have in clinical practice. The current guidelines identify 3 risk groups, where only hs-cTn concentrations at the limit of detection or significantly above the 99th percentile clearly triage patients toward rule-out or rule-in of AMI, respectively.[6] This outcome leaves a significant proportion of patients within the observe zone of clinical uncertainty requiring repeat cTn measurement and further investigation.[32] In the current study, of the patients who ultimately did not have AMI, the proportion in the observe zone after the first measurement at presentation was 55.2% using hs-cTnT, 63.2% using hs-cTnI, and 46.1% using cMyC. It is expected that the greater diagnostic certainty afforded on a single-presentation blood draw by cMyC may reduce median time to discharge and costs of investigations.

As yet, near-patient, point-of-care devices have not been able to rule out AMI because they have struggled to achieve the required analytic sensitivity to measure low concentrations of cTnT or cTnI. In addition, the development of reliable large platform-based hs-cTn assays has proved more challenging than expected. Until now, only 2 manufacturers have overcome the difficulties of developing and introducing hs-cTn assays into clinical practice,[6] of which 1 had major quality issues initially.[33–35] These uncertainties and concerns have led to delays in the approval of these assays for clinical care in the United States.[36] The US Food and Drug Administration has only recently ratified the use of the fifth-generation hs-cTnT assay.[37] Because cMyC is more abundant and rises more rapidly, migration to a point-of-care format may be less challenging. Risk prediction appears grossly similar when comparing hs-cTn and cMyC and could therefore be performed on either. Notably, a cMyC level <10 ng/L (the threshold resembling 25 times the limit of detection) offers both high negative predictive value and 30-day mortality rates approaching 0.

Our study has a number of limitations. First, the diagnostic cutoffs for cMyC require external validation. Despite its size, a single cohort cannot entirely safeguard against calibration issues and is inherently subject to potential institutional bias. We have attempted to mitigate these risks by using both randomization and bootstrapping, but in an ideal scenario the findings require validation in an independent cohort. Second, the analyses within this manuscript are confined to the concentration of the necrosis biomarker on first blood draw. We have not analyzed the effect on the gray zone of repeat blood draws after set intervals. This area of active research has no consensus regarding resampling interval, magnitude of concentration change, use of absolute or relative change in concentration, or effect of assay vendor.[4,5,21,38–40] Third, as a prospective diagnostic study, we cannot exactly quantify the clinical benefit associated with the use of cMyC as an alternative or addition to hs-cTn. Further cluster-randomized studies will be required to address this issue. Fourth, we cannot comment on the accuracy of cMyC among patients with terminal kidney failure on renal replacement therapy or ST elevation myocardial infarction because such patients were excluded from this study. Currently, biomarkers have no role in the assessment of patients with ST elevation myocardial infarction, and hence this group was not analyzed. Fifth, of 3029 patients recruited, 875 had no baseline cMyC measured. However, a comparison between the analyzed cohort and the excluded patient sample has not demonstrated substantial differences in baseline characteristics (Table III in the online-only Data Supplement). Sixth, in patients with low levels of cMyC (eg, the noncardiac chest pain group), we observed a significant difference in biomarker values dependent on certain underlying conditions (such as prior coronary artery disease) (Tables VII and VIII in the online-only Data Supplement). However, this effect is muted in patients with AMI and indeed did not negatively influence specificity. Finally, cMyC was measured using a research platform, whereas hs-cTnI and hs-cTnT were measured using widely available clinical laboratory analyzers. The sandwich immunoassay is comparable to the setup used to test for hs-cTn, but cMyC is not yet available on a random-access laboratory analyzer for routine clinical use.

In summary, cMyC is a promising new biomarker of myocardial necrosis, with overall discriminatory power comparable with the leading troponin assays in AMI diagnosis. A potential advantage of cMyC is its ability to more effectively rule out AMI at presentation, particularly among those presenting early after symptom onset.

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DISCLOSURES

Dr Twerenbold has received a research grant from the Swiss National Science Foundation (P300PB-167803) and speaker/consulting honoraria from Roche, Abbott, and BRAHMS. Dr Rubini has received speaker honoraria from Abbott. Dr Reichlin has received research grants from Goldschmidt-Jacobson-Foundation, the Swiss National Science Foundation (PASMP3-136995), the Swiss Heart Foundation, the University of Basel, the Professor Max Cloetta Foundation, the Uniscientia Foundation Vaduz, and the Department of Internal Medicine, University Hospital Basel; and speaker honoraria from BRAHMS and Roche. Dr Mueller has received research grants from the Swiss National Science Foundation, the Swiss Heart Foundation, the European Union, the Swiss Commission for Technology and Innovation, the Cardiovascular Research Foundation Basel, the University Hospital Basel, Abbott, Alere, Astra Zeneca, Beckman Coulter, BG Medicine, Biomerieux, BRAHMS, Critical Diagnostics, Nanosphere, Roche, Siemens, Singulex, Sphingotec, Department of Internal Medicine (University Hospital Basel), and 8sense; and speaker/consulting honoraria from Abbott, Alere, Astra Zeneca, Biomerieux, BMS, Boehringer Ingelheim, BRAHMS, Cardiorentis, Duke University, Eli Lilly, Novartis, Radiometer, Roche, Sanofi, Siemens, and Singulex. The other authors report no conflicts of interest. Millipore Sigma was contracted to undertake the analyses of cMyC on a fee-for-service basis and holds no commercial interest. Prof Marber is named as an inventor on a patent held by King's College London for the detection of cMyC as a biomarker of myocardial injury.

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FOOTNOTES

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Direct Comparison of Cardiac Myosin-Binding Protein C With Cardiac Troponins for the Early Diagnosis of Acute Myocardial Infarction

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Correction to: Direct Comparison of Cardiac Myosin-Binding Protein C With Cardiac Troponins for the Early Diagnosis of Acute Myocardial Infarction

In the article by Kaier et al, "Direct Comparison of Cardiac Myosin-Binding Protein C With Cardiac Troponins for the Early Diagnosis of Acute Myocardial Infarction," which which published ahead of print September 26, 2017, and appeared in the October 17, 2017, issue of the journal (*Circulation*. 2017;136:1495–1508), errors appeared in Tables 3 and 4.

Under Thresholds, in column 4, the rows should each read "Specificity" instead of "Sensitivity" in both Tables 3 and 4.

The correction has been made to the current online version of the article, which is available at http://circ.ahajournals.org/content/136/16/1495.

Circulation is available at http://circ.ahajournals.org.

Supplemental Material: Direct comparison of cardiac myosin-binding protein C with cardiac troponins for the early diagnosis of acute myocardial infarction

Running title: Cardiac Myosin-binding protein C in the diagnosis of AMI

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Supplemental Methods

Routine clinical assessment

All patients underwent a clinical assessment that included medical history, physical examination, 12-lead ECG, pulse oximetry, standard blood test, and chest radiography according to local protocols and in accordance with the guidelines of the European Society of Cardiology (ESC).[1] Levels of cTn were measured at presentation and serially thereafter as long as clinically indicated. Treatment of patients was left to discretion of the attending physician.

Adjudication of the final diagnosis

AMI was defined and cTn levels interpreted as recommended in current guidelines.[2-5] In brief, AMI was diagnosed when there was evidence of myocardial necrosis with a significant rise and/or fall in a clinical setting consistent with myocardial ischemia. Patients with AMI were further subdivided into type 1 AMI (primary coronary events) and type 2 AMI (ischemia due to increased demand or decreased supply, for example tachyarrhythmia or hypertensive crisis).[2,6]

The adjudication of final diagnoses was performed centrally in the core lab (University Hospital Basel) for all patients incorporating levels of hs-cTnT (see test characteristics above). More specifically, two independent cardiologists not directly involved in patient care reviewed all available medical records (including patient history, physical examination, results of laboratory testing including hs-cTnT levels, radiologic testing, ECG, echocardiography, cardiac exercise test, lesion severity and morphology in coronary angiography, discharge summary) pertaining to the patient from the time of ED presentation to 90-day follow-up. Late samples were available for adjudication of final diagnosis in all patients. In general, serial sampling was performed until at least 6h after presentation to the ED or onset of chest.[6] In situations of diagnostic disagreement, cases were reviewed and adjudicated in conjunction with a third cardiologist. While discharge diagnoses often were correct and in agreement with the final adjudicated diagnosis, there were also cases where those diagnoses needed to be revised, most often because more information became available from medical testing during early follow-up, and more rarely, because the discharge diagnosis was not in agreement with the Universal Definition of AMI.

The 99th percentile (14ng/L) was used as cut-off for myocardial necrosis. Absolute cTn changes were used to determine significant changes based on the diagnostic superiority of absolute over relative changes.[7-12] Based on studies of the biological variation of cTn {Wu:2008bf, Vasile:2010fc} as well as on data from previous chest pain cohort studies {Reichlin:2011iu, Hammarsten:2012cv}, a significant absolute change was defined as a rise or fall of at least 10ng/L within six hours, or, in an assumption of linearity, as an absolute change of 6ng/L within three hours. Predefined alternative diagnoses included “unstable angina” (UA), “Cardiac symptoms of origin other than coronary artery disease” and “non-cardiac chest pain”.

Clinical Care: The (hs)-cTn assays and cut-off levels used for local clinical care

Routine clinical care comprised five different cTn assays at the different hospitals and at the different recruitment periods. The cTn assays used clinically in most of the participating institutions changed during the study from a conventional cTn assay to the hs-cTnT assay. In order to take advantage of the higher sensitivity and higher overall diagnostic accuracy offered by the hs-cTnT assay, patients were adjudicated using the hs-cTnT values in all patients. In patients in whom clinically a conventional cTn assay was used, the conventional cTn values and the hs-cTnT values were available for the adjudication. In patients in whom clinically the hs-cTnT assay was used, only the hs-cTnT values were available for the adjudication.

The following conventional cTn assays were used: For the Roche cTnT 4th generation assay, the 10% CV level is 0.035ug/l. The laboratories of the participating sites reported only two decimals; therefore 0.04ug/l was used as a cut-off for myocardial necrosis. In order to fulfil the criteria of a significant change (30% of 99th percentile or 10% CV level), a patient would e.g. need to have a level of <0.01ug/l at presentation and 0.04ug/l at 6h. A patient would also qualify if the first level is 0.02ug/l and the second 0.04ug/l. A patient would not fulfil the criteria if the first level is 0.03ug/l and the second is 0.04ug/l. If the first level is 0.04ug/l, the second level needs to be at least 0.06ug/l.

For the Abbott Axsym cTnI ADV, the 10% CV level is 0.16ug/l. A patient having 0.16ug/l at presentation would meet the criteria for significant change if the second was ≥0.21ug/l. A patient having <0.12ug/l at presentation (limit of detection) would qualify if the second is >0.16ug/l.

For the Beckmann Coulter Accu cTnI, the 10% CV level is 0.06ug/l. A patient having 0.06ug/l at presentation would qualify if the second is ≥0.08ug/l. A patient having 0.05 at presentation would qualify if the second is 0.07ug/l, but not 0.06ug/l. A patient having undetectable cTnI (cTnI<0.01ug/l) at presentation would qualify if the second is ≥0.06ug/l.

For the Siemens Dimension Vista s-cTnI, the 10% CV level is 40ng/L. The limit of detection is 15ng/L and the 99th percentile is 45ng/L. An absolute change of 20ng/L or more within 3-6h was considered significant.

For Elecsys hs-cTnT measured clinically, the same change criteria were applied as for hs-cTnT measured from the study blood samples.

Central adjudication: Definition of rise and/or fall in high-sensitivity cardiac troponin T (hs-cTnT)

Absolute changes in hs-cTnT were used to determine significant changes based on the diagnostic superiority of absolute over relative changes.[7-12] Based on studies of the biological variation of cTn [13,14] as well as on data from previous chest

pain cohort studies [15,16], a significant absolute change was defined as a rise or fall of at least 10 ng/L within 6 hours or an absolute change of 6 ng/L within 3 hours. If later clinical samples (e.g., at 24, 48, or 72 hours) revealed a lower hs-cTnT level than that measured during the period of sampling in the ED, the later level was considered the true baseline level for the calculation of the change criteria.

Measurement of high-sensitivity cardiac troponin I, high-sensitivity cardiac troponin T and sensitive cardiac troponin I

After collection and subsequent centrifugation, samples were frozen at -80ºC until assayed in a blinded fashion in a dedicated core laboratory. The Roche hs-cTnT assay was measured on the Elecsys 2010 (Roche Diagnostics). The limit of blank and LoD were determined to be 3 and 5 ng/L, respectively. The 99th-percentile of a healthy reference population was reported at 14 ng/L with an imprecision corresponding to 10% CV at 13 ng/L.[17] This study does not include any measurements with hs-cTnT lots that required the revision of the calibration curve.[18-22] The Abbott hs-cTnI assay used was the final pre-commercial release version of the ARCHITECT High Sensitive STAT Troponin I assay (Abbott Laboratories, Abbott Park, IL, USA). Samples were thawed, mixed, and centrifuged (for 30 min at 3000 RCF and 4ºC for serum samples or for 10 min, twice, at 3000 RCF for plasma samples) prior to analysis and according to manufacturer's instructions. The hs-cTnI assay has a 99th percentile concentration of 26·2 ng/L with a corresponding coefficient of variation (CV) of <5% and a limit of detection (LoD) of 1.9 ng/L.[23] The cTnI-ultra assay was performed with the use of the ADVIA Centaur immunoassay system (Siemens). Limit of detection is 6 ng/L; a 10% coefficient of variation was reported at 30 ng/L with the 99th percentile cut-off point of 40 ng/L.[24,25] Calculation of the glomerular filtration rate was performed using the abbreviated Modification of Diet in Renal disease formula.[26]

Measurement of cardiac myosin-binding protein C

We have previously described the creation, biophysical selection and organ specificity of mouse monoclonal antibodies recognising cardiac-restricted epitopes within the N-terminus of cMyC.[27] Two of these antibodies, 1A4 and 3H8, were used to create a sensitive sandwich immunoassay. In brief, Magnetic microparticles (MPs) for capture were prepared by binding 25µg of mouse monoclonal (1A4) per mg of MPs. The coated MPs were diluted in assay buffer (proprietary mix with custom 450mM NaCl and 0.5% Triton X-100) to 100µg/mL. Due to sample volume constraints, serum, plasma or analyte (recombinant C0C2 domain of cMyC)[27] were diluted 2.2 fold with standard diluent and 100µL added per well of a 96-well assay plate. Samples or standards were then exposed to 100µL of coated MPs and agitated for 2 hours at 25°C. MPs were retained via a magnetic bed with unbound material removed in a single wash step. Fluorescently-labelled mouse monoclonal (3H8) detection antibody was diluted in assay buffer to 100ng/mL. To each well, 20µL of detection antibody was added and the MPs agitated for 1 hour at 25°C, retained via a magnetic bed and then washed 4 times to

remove any unbound detection reagent. The MPs were then transferred to a new plate and all buffer was aspirated. The MPs were then exposed to 20µL/well of elution buffer B for 5 minutes at 25°C before transfer to a 384-well plate containing 10µL/well of neutralization buffer D. Fluorescent label was then detected by single molecule counting using the Erenna system with a dwell time of 60s per well. Three signal outputs were obtained from the Erenna System: Detected Events (DEs; low end signal), Event Photons (EPs; low end and higher end signal), and Total Photons (TPs; high end signal).

Supplemental Tables

Table S1. Comparison of biomarkers in patients excluded because of uncertain final diagnosis (e.g. patients discharged based on negative result on conventional cTn assay, who then tested positive on high-sensitivity cTn assay); comparison is performed for all patients with a measured cMyC at baseline (N=60) and all patients including missing values (N=92)

Biomarker	N	Median ng/L [IQR]
cMyC at 0h	60	36 [24-62]
hs-cTnI at 0h	56	11 [7-21]
hs-cTnT at 0h	60	21 [16-28]
All patients	92	
cMyC at 0h	60	36 [24-62]
hs-cTnI at 0h	78	11 [6-19]
hs-cTnT at 0h	92	22 [17-29]

Table S2. Demographics: group qualifying for primary analysis (n=1954) vs patients excluded due to missing cMyC values at baseline

Demographics	**All patients (n = 1954)**	**Excluded patients (n=875)**	**p value*** for comparison
Age, years	62 ± 16	59 ± 16	<0.001
Male	1341 (69)	587 (67)	0.441
Risk factors			
Hypertension	1247 (64)	384 (44)	<0.001
Hyperlipidaemia	992 (51)	421 (48)	0.206
Diabetes mellitus	348 (18)	136 (16)	0.155
Current smoking	476 (24)	244 (28)	0.051
History of smoking	1194 (61)	553 (63)	0.297
History			
Coronary artery disease	710 (36)	272 (31)	0.008
Previous myocardial infarction	474 (24)	199 (23)	0.408
Previous revascularisation (CABG or PCI)	553 (28)	237 (27)	0.535
Peripheral artery disease	119 (6)	52 (6)	0.947
Previous stroke	100 (5)	53 (6)	0.352
Vital status			
Heart rate, beats/min	79 ± 20	81 ± 21	0.234
Systolic blood pressure, mm Hg	144 ± 24	143 ± 25	0.711
Diastolic blood pressure, mm Hg	82 ± 15	82 ± 15	0.569
Electrocardiographic findings			
ST-segment depression	193 (10)	75 (9)	0.313
T-wave inversion	260 (13)	89 (10)	0.026
No significant electrocardiographic abnormalities	1469 (75)	681 (79)	0.193
Laboratory assessment			
Estimated glomerular filtration rate, ml/min/1.73m2†	84 ± 26	87 ± 25	0.008
Presentation time			
Time since chest pain onset, hours	5 [3, 12]	4 [1, 9]	<0.001
Time since chest pain peak, hours	3 [2, 7]	2 [5, 5]	<0.001

Legend: * p values for comparison included versus excluded patient groups; data are expressed as medians [1st quartile, 3rd quartile] or means ± standard deviation, for categorical variables as numbers (percentages); CABG = Coronary Artery Bypass Graft; PCI = Percutaneous Coronary Intervention; † glomerular filtration rate was estimated using the Modification of Diet in Renal Disease (MDRD) formula

Table S3. STARD checklist for studies of diagnostic accuracy

Section & Topic	No	Item	Reported on page #
TITLE OR ABSTRACT	1	Identification as a study of diagnostic accuracy using at least one measure of accuracy (such as sensitivity, specificity, predictive values, or AUC)	1, 3
ABSTRACT	2	Structured summary of study design, methods, results, and conclusions (for specific guidance, see STARD for Abstracts)	3-4
INTRODUCTION	3	Scientific and clinical background, including the intended use and clinical role of the index test	5-6
	4	Study objectives and hypotheses	5-6
METHODS			
Study design	5	Whether data collection was planned before the index test and reference standard were performed (prospective study) or after (retrospective study)	7
Participants	6	Eligibility criteria	7
	7	On what basis potentially eligible participants were identified (such as symptoms, results from previous tests, inclusion in registry)	7
	8	Where and when potentially eligible participants were identified (setting, location and dates)	7-8
	9	Whether participants formed a consecutive, random or convenience series	7
Test methods	10a	Index test, in sufficient detail to allow replication	8-9 and supplement
	10b	Reference standard, in sufficient detail to allow replication	8-9 and supplement
	11	Rationale for choosing the reference standard (if alternatives exist)	n/a
	12a	Definition of and rationale for test positivity cut-offs or result categories of the index test, distinguishing pre-specified from exploratory	9-10
	12b	Definition of and rationale for test positivity cut-offs or result categories of the reference standard, distinguishing pre-specified from exploratory	9-10
	13a	Whether clinical information and reference standard results were available to the performers/readers of the index test	7-9
	13b	Whether clinical information and index test results were available to the assessors of the reference standard	7-9
Analysis	14	Methods for estimating or comparing measures of diagnostic accuracy	8-10
	15	How indeterminate index test or reference standard results were handled	8-10
	16	How missing data on the index test and reference standard were handled	9
	17	Any analyses of variability in diagnostic accuracy, distinguishing pre-specified from exploratory	9
	18	Intended sample size and how it was determined	n/a
RESULTS			
Participants	19	Flow of participants, using a diagram	7 and Figures S1 + 2S
	20	Baseline demographic and clinical characteristics of participants	13, table 1

	21a	Distribution of severity of disease in those with the target condition	13, figure 2, suppl table 3S
	21b	Distribution of alternative diagnoses in those without the target condition	13, table 1
	22	Time interval and any clinical interventions between index test and reference standard	n/a
Test results	23	Cross tabulation of the index test results (or their distribution) by the results of the reference standard	13-16, tables 2+3, figures 3A+3B, suppl tables 4S+5S
	24	Estimates of diagnostic accuracy and their precision (such as 95% confidence intervals)	14
	25	Any adverse events from performing the index test or the reference standard	14-15
DISCUSSION			
	26	Study limitations, including sources of potential bias, statistical uncertainty, and generalisability	20
	27	Implications for practice, including the intended use and clinical role of the index test	2, 18-21
OTHER INFORMATION			
	28	Registration number and name of registry	4, 7
	29	Where the full study protocol can be accessed	7
	30	Sources of funding and other support; role of funders	22-24

Table S4. Demographics for derivation and validation cohorts

Demographics	**All patients (n = 1954)**	**Derivation (n = 586)**	**Validation (n = 1368)**	**p value*** for comparison
Age, years	62 ± 16	62 ± 16	62 ± 16	0.777
Male	1341 (69)	393 (67)	948 (69)	0.357
Acute Myocardial Infarction	340 (17)	107 (18)	233 (17)	0.512
Risk factors				
Hypertension	1247 (64)	362 (62)	885 (65)	0.239
Hyperlipidaemia	992 (51)	290 (49)	702 (51)	0.489
Diabetes mellitus	348 (18)	99 (17)	249 (18)	0.505
Current smoking	476 (24)	148 (25)	328 (24)	0.602
History of smoking	1194 (61)	372 (63)	864 (63)	0.906
History				
Coronary artery disease	710 (36)	200 (34)	510 (37)	0.202
Previous myocardial infarction	474 (24)	136 (23)	338 (25)	0.515
Previous revascularisation (CABG or PCI)	553 (28)	153 (26)	400 (29)	0.176
Peripheral artery disease	119 (6)	33 (6)	86 (6)	0.652
Previous stroke	100 (5)	27 (5)	73 (5)	0.577
Vital status				
Heart rate, beats/min	79 ± 20	80 (20)	79 (21)	0.895
Systolic blood pressure, mm Hg	144 ± 24	145 ± 25	143 ± 24	0.058
Diastolic blood pressure, mm Hg	82 ± 15	82 ± 15	82 ± 15	0.765
Electrocardiographic findings				
ST-segment depression	193 (10)	53 (9)	140 (10)	0.475
T-wave inversion	260 (13)	64 (11)	196 (14)	0.05
No significant electrocardiographic abnormalities	1469 (75)	456 (80)	1013 (76)	0.075
Laboratory assessment				
Estimated glomerular filtration rate, ml/min/1.73m2†	84 ± 26	84 ± 25	84 ± 26	0.441
Presentation time				
Time since chest pain onset, hours	5 [3, 12]	5 [2, 12]	5 [3, 12]	0.804
Time since chest pain peak, hours	3 [2, 7]	4 [2, 7]	3 [2, 7]	0.528

Legend: * p values for comparison validation to derivation cohort; data are expressed as medians [1st quartile, 3rd quartile] or means ± standard deviation, for categorical variables as numbers (percentages); CABG = Coronary Artery Bypass Graft; PCI = Percutaneous Coronary Intervention; † glomerular filtration rate was estimated using the Modification of Diet in Renal Disease (MDRD) formula

Table S5. Blood concentrations of cMyC, hs-cTnT, hs-cTnI and s-cTnI at presentation in the five diagnostic categories

Adjudicated diagnosis	cMyC (ng/L)	hs-cTnT (ng/L)	hs-cTnI (ng/L)	s-cTnI (mg/L)
AMI	237 [71-876]	62 [28-139]	97 [21-456]	0.175 [0.039-0.722]
Unstable angina	21 [13-43]	11 [7-17]	6 [4-12]	0.009 [0.005-0.020]
cardiac symptoms of origin other than coronary artery disease	33 [12-96]	15 [7-32]	10 [4-30]	0.017 [0.005-0.044]
non-cardiac symptoms	10 [6-19]	6 [4-10]	3 [2-5]	0.005 [0.004-0.011]
symptoms of unknown origin	11 [7-16]	6 [3-10]	3 [2-5]	0.005 [0.001-0.010]

Legend: AMI = acute myocardial infarction; data is quoted in median ng/L [Interquartile Range] for cMyC and hs-cTn assays, and mg/L [IQR] for s-cTnI

Table S6. Blood concentrations of biomarkers above 99th centiles at presentation

cMyC >87 ng/L	**AMI**	**UA**	**non-coronary**	**non-cardiac**	**unknown**	**p**	**N**
	N=237	N=18	N=72	N=22	N=1		
cMyC at 0h	559 [215-1228]	135 [113-207]	168 [120-329]	157 [101-222]	96 [96-96]	<0.001	350
hs-cTnI at 0h	230 [74-725]	42 [17-74]	81 [34-227]	29 [15-43]	3 [3-3]	<0.001	327
hs-cTnT at 0h	92 [51-182]	29 [24-43]	50 [35-88]	26 [19-47]	8 [8-8]	<0.001	337
adjusted R^2: cMyC and hs-cTnI 0.230, cMyC and hs-cTnT 0.504, hs-cTnT and hs-cTnI 0.608							

hs-cTnI >26 ng/L	**AMI**	**UA**	**non-coronary**	**non-cardiac**	**unknown**	**p**	**N**
	N=226	N=21	N=67	N=30	N=0		
cMyC ng/L at 0h	524 [208-1230]	60 [30-134]	158 [99-344]	75 [36-167]	NA	<0.001	344
hs-cTnI ng/L at 0h	235 [84-739]	60 [53-98]	91 [49-229]	42 [30-65]	NA	<0.001	344
hs-cTnT ng/L at 0h	93 [54-183]	22 [14-32]	50 [35-88]	27 [15-47]	NA	<0.001	332
adjusted R^2: cMyC and hs-cTnI 0.230, cMyC and hs-cTnT 0.528, hs-cTnT and hs-cTnI 0.602							
hs-cTnT >14 ng/L	AMI	UA	non-coronary	non-cardiac	unknown	p	N
	N=290	N=63	N=135	N=150	N=0		
cMyC ng/L at 0h	328 [97-998]	48 [24-83]	91 [46-165]	34 [19-57]	NA	<0.001	638
hs-cTnI ng/L at 0h	134 [33-557]	14 [7-38]	28 [14-87]	10 [6-19]	NA	<0.001	600
hs-cTnT ng/L at 0h	70 [36-147]	21 [17-26]	31 [20-52]	20 [16-27]	NA	<0.001	638
adjusted R^2: cMyC and hs-cTnI 0.274, cMyC and hs-cTnT 0.567, hs-cTnT and hs-cTnI 0.617							

Legend: AMI = acute myocardial infarction; UA = unstable angina; data is quoted in median ng/L [Interquartile Range]

Table S7. Non-Cardiac sources of cMyC variation

	AMI group (N=340)			**p**	**N**
Gender - male vs female	207 [62-814]	vs	361 [91-1006]	0.096	256
Age - <65 vs ≥65	237 [62-938]	vs	237 [74-826]	0.925	122
Body Mass Index (BMI) - <30 vs ≥30	264 [72-898]	vs	219 [76-616]	0.414	257
Hypertension - absent vs present	272 [64-885]	vs	230 [73-874]	0.935	71
Hyperlipidaemia - absent vs present	321 [92-840]	vs	211 [57-887]	0.140	113
Diabetes mellitus - absent vs present	282 [75-1004]	vs	182 [64-535]	0.059	245
Current smoking - absent vs present	257 [66-894]	vs	213 [78-822]	0.681	249
History of smoking - absent vs present	219 [70-837]	vs	274 [72-894]	0.701	198
Coronary artery disease - absent vs present	308 [79-973]	vs	206 [60-785]	0.135	166
Estimated glomerular filtration rate, ml/min/1.73m2* - <60 vs ≥60	345 [87-953]	vs	208 [60-828]	0.111	101
	Non-cardiac chest pain group (N=1052)				
Gender – male vs female	10 [6-19]	vs	10 [5-18]	0.108	716
Age - <65 vs ≥65	8 [5-13]	vs	18 [11-32]	<0.001	701
Body Mass Index (BMI) - <30 vs ≥30	10 [6-19]	vs	11 [6-21]	0.282	815
Hypertension – absent vs present	7 [5-12]	vs	14 [9-29]	<0.001	509
Hyperlipidaemia – absent vs present	8 [5-15]	vs	14 [8-28]	<0.001	634
Diabetes mellitus – absent vs present	10 [6-17]	vs	16 [10-35]	<0.001	916
Current smoking – absent vs present	11 [7-21]	vs	8 [5-14]	<0.001	769
History of smoking – absent vs present	10 [6-17]	vs	13 [7-23]	<0.001	707
Coronary artery disease – absent vs present	9 [6-15]	vs	18 [11-32]	<0.001	784
Estimated glomerular filtration rate, ml/min/1.73m2* - <60 vs ≥60	30 [16-53]	vs	9 [6-16]	<0.001	107

Legend: MI = myocardial infarction, based on the adjudicated gold-standard diagnosis; CABG = Coronary Artery Bypass Graft; PCI = Percutaneous Coronary Intervention; data is quoted in median [Interquartile Range]; N = number of patients with the condition on the left-hand side of the demographic factors (e.g. 'Hypertension – absent in 509 patients'); *glomerular filtration rate was estimated using the Modification of Diet in Renal Disease (MDRD) formula

Table S8. Multiple regression to determine influence of baseline variables on cMyC levels

Non-cardiac chest pain group	R^2	B	SE B	β_i	p
	0.077				
Constant		-32.439	11.893		0.006
Hypertension		2.040	3.860	0.020	0.597
Hyperlipidaemia		1.326	4.127	0.013	0.748
Diabetes mellitus		1.139	5.047	0.007	0.822
Current smoking		-1.914	3.978	-0.017	0.631
History of smoking		-3.531	3.680	-0.033	0.338
Coronary artery disease		9.658	4.552	0.083	0.034
Creatinine on admission		0.357	0.072	0.157	0.000
Age		0.399	0.113	0.127	0.000
Body Mass Index (BMI)		-0.007	0.328	-0.001	0.982
	0.075				
Constant		-34.087	7.050		0.000
Coronary artery disease		10.680	3.662	0.093	0.004
Creatinine on admission		0.351	0.070	0.155	0.000
Age		0.428	0.099	0.137	0.000

AMI group	R^2	B	SE B	β_i	p
	0.028				
Constant		516.589	496.148		0.299
Hypertension		-1.724	121.878	-0.001	0.989
Hyperlipidaemia		141.837	106.276	0.082	0.183
Diabetes mellitus		-236.237	108.644	-0.129	0.030
Current smoking		-30.010	136.079	-0.016	0.826
History of smoking		15.745	107.937	0.010	0.884
Coronary artery disease		-175.913	102.811	-0.108	0.088
Creatinine on admission		0.727	0.933	0.045	0.436
Age		-0.459	4.339	-0.007	0.916
Body Mass Index (BMI)		4.402	11.964	0.023	0.713
	0.014				
Constant		674.182	52.552		0.000
Diabetes mellitus		-220.143	100.580	-0.119	0.029

Legend: AMI = Acute Myocardial Infarction; R^2 = fit of the regression model; B = beta estimate; SE B = standard errors of beta estimate; β_i = standardized beta estimate

Table S9. Derivation cohort – hs-cTnT

Initial model	New model - cMyC (10/120) - Derivation cohort					
hs-cTnT	No AMI (n=465)			AMI (n=103)		
	Rule-out	Observe	Rule-in	Rule-out	Observe	Rule-in
Rule-out	105	28	0	0	0	0
Observe	95	221	8	0	41	10
Rule-in	0	0	8	0	3	49
NRI	0.127 (95% CI, 0.061-0.173)			0.068 (95% CI, 0.0-0.136)		
NRI (dimensionless)	0.195 (95% CI, 0.113-0.277); p value <0.001			IDI	0.065 (95% CI, 0.037-0.093)	
Thresholds	**Sensitivity** (95% CI)		**NPV** (95% CI)	**Specificity** (95% CI)		**PPV** (95% CI)
hs-cTnT 5 ng/L	100% (100-100%)		100% (100-100%)	28.8% (24.8-33.1%)		23.8% (19.4-27.8%)
hs-cTnT 52 ng/L	50.5% (41.3-60.4%)		89.9% (87.4-92.6%)	98.3% (97-99.3%)		86.9% (77.2-94.1%)
cMyC 10 ng/L	100% (100-100%)		100% (100-100%)	41.3% (36.8-45.9%)		27.3% (22.9-31.9%)
cMyC 120 ng/L	57.1% (47.5-67%)		91.1% (88.5-93.6%)	96.6% (94.8-98.1%)		78.9% (68.8-87.6%)

Legend: NRI = Net Reclassification Improvement; IDI = Integrated Discrimination Improvement; CI = Confidence Interval; NPV = Negative Predictive Value; PPV = Positive Predictive Value; AMI = Acute Myocardial Infarction, based on the adjudicated gold-standard diagnosis

Table S10. Derivation cohort – hs-cTnI

Initial model	New model - cMyC (10/120) – Derivation cohort					
hs-cTnI	No AMI (n=457)			AMI (n=96)		
	Rule-out	Observe	Rule-in	Rule-out	Observe	Rule-in
Rule-out	61	10	2	0	0	0
Observe	141	224	3	0	37	5
Rule-in	1	4	11	0	6	48
NRI	0.287 (95% CI, 0.217-0.336)			-0.010 (95% CI, -0.081-0.060)		
NRI (dimensionless)	0.276 (95% CI, 0.191-0.361); p value <0.001			IDI	0.090 (95% CI, 0.062-0.119)	
Thresholds	**Sensitivity** (95% CI)		**NPV** (95% CI)	**Specificity** (95% CI)		**PPV** (95% CI)
hs-cTnI 2 ng/L	100% (100-100%)		100% (100-100%)	15.9% (12.7-19.3%)		20% (16.3-23.8%)
hs-cTnI 52 ng/L	55.9% (46.2-66%)		91.3% (88.8-93.7%)	96.5% (94.9-98%)		77.3% (66.7-86.4%)
cMyC 10 ng/L	100% (100-100%)		100% (100-100%)	42.7% (38.3-47.4%)		26.9% (22.2-31.1%)
cMyC 120 ng/L	55.3% (45.8-64.8%)		91.2% (88.5-93.6%)	96.5% (94.8-98%)		77.1% (66.7-86.1%)

Legend: NRI = Net Reclassification Improvement; IDI = Integrated Discrimination Improvement; CI = Confidence Interval; NPV = Negative Predictive Value; PPV = Positive Predictive Value; AMI = Acute Myocardial Infarction, based on the adjudicated gold-standard diagnosis

Table S11. Net Reclassification Improvement – Onset of chest pain <3 hours prior to presentation

Initial model	New model - MyC (10/120) - chest pain for <3hrs					
hs-cTnT	No AMI (n=382)			AMI (n=78)		
	Rule-out	Observe	Rule-in	Rule-out	Observe	Rule-in
Rule-out	99	28	0	0	1	0
Observe	83	161	6	0	38	10
Rule-in	0	0	5	0	1	28
NRI	0.128 (95% CI, 0.055-0.181)			0.128 (95% CI, 0.044-0.213)		
NRI (dimensionless)	0.256 (95% CI, 0.157-0.356); p value <0.001			IDI	0.086 (95% CI, 0.052-0.119)	
Thresholds	**Sensitivity** (95% CI)		**NPV** (95% CI)	**Specificity** (95% CI)		**PPV** (95% CI)
hs-cTnT 5 ng/L	98.8% (95.8-100%)		99.2% (97.5-100%)	33.3% (28.6-38.1%)		23.3% (19-27.9%)
hs-cTnT 52 ng/L	37.2% (25.9-48.2%)		88.5% (85.3-91.4%)	98.7% (97.5-99.7%)		86.1% (73.3-96.9%)
cMyC 10 ng/L	100% (100-100%)		100% (100-100%)	46.4% (41.5-51.6%)		27.5% (22.3-32.5%)
cMyC 120 ng/L	49% (36.8-60%)		90.4% (87.4-93.2%)	97.2% (95.3-98.7%)		77.8% (65.2-89.7%)

Initial model	New model - MyC (10/120) - chest pain for <3hrs					
hs-cTnI	No AMI (n=381)			AMI (n=79)		
	Rule-out	Observe	Rule-in	Rule-out	Observe	Rule-in
Rule-out	76	11	1	0	0	0
Observe	109	169	4	0	39	8
Rule-in	0	5	6	0	4	28
NRI	0.257 (95% CI, 0.185-0.310)			0.051 (95% CI, -0.032-0.133)		
NRI (dimensionless)	0.308 (95% CI, 0.210-0.406); p value <0.001			IDI	0.101 (95% CI, 0.067-0.135)	
Thresholds	**Sensitivity** (95% CI)		**NPV** (95% CI)	**Specificity** (95% CI)		**PPV** (95% CI)
hs-cTnI 2 ng/L	100% (100-100%)		100% (100-100%)	23.2% (19-27.1%)		21.3% (16.8-25.7%)
hs-cTnI 52 ng/L	40.3% (29.5-51.2%)		88.7% (85.5-91.7%)	97.2% (95.5-98.7%)		74.8% (60.7-87.5%)
cMyC 10 ng/L	100% (100-100%)		100% (100-100%)	47.1% (42.4-52.2%)		28.2% (22.9-33.3%)
cMyC 120 ng/L	45.6% (34.7-57.3%)		89.6% (86.7-92.6%)	97.1% (95.3-98.7%)		76.6% (64.1-87.8%)

Legend: NRI = Net Reclassification Improvement; IDI = Integrated Discrimination Improvement; CI = Confidence Interval; NPV = Negative Predictive Value; PPV = Positive Predictive Value; AMI = Acute Myocardial Infarction, based on the adjudicated gold-standard diagnosis

Table S12. Net Reclassification Improvement – Onset of chest pain ≥3 hours prior to presentation

Initial model	**New model - MyC (10/120) - chest pain for ≥3hrs**					
hs-cTnT	No AMI (n=1172)			AMI (n=244)		
	Rule-out	Observe	Rule-in	Rule-out	Observe	Rule-in
Rule-out	255	77	0	0	0	0
Observe	202	569	34	1	69	24
Rule-in	0	7	28	0	11	139
NRI	0.084 (95% CI, 0.034-0.114)			0.049 (95% CI, 0.0-0.098)		
NRI (dimensionless)	0.133 (95% CI, 0.076-0.190); p value <0.001			IDI	0.044 (95% CI, 0.025-0.063)	
Thresholds	**Sensitivity** (95% CI)		**NPV** (95% CI)	**Specificity** (95% CI)		**PPV** (95% CI)
hs-cTnT 5 ng/L	100% (100-100%)		100% (100-100%)	28.4% (25.8-31%)		22.6% (20.1-25.1%)
hs-cTnT 52 ng/L	61.4% (55.6-67.3%)		92.3% (90.9-93.9%)	97% (96-97.9%)		81.1% (75.3-86.7%)
cMyC 10 ng/L	99.6% (98.7-100%)		99.8% (99.3-100%)	37.3% (34.8-40.3%)		24.9% (22-27.8%)
cMyC 120 ng/L	66.9% (61-72.6%)		93.2% (91.8-94.5%)	94.7% (93.4-96%)		72.5% (66.7-78.1%)

Initial model	New model - MyC (10/120) - chest pain for ≥3hrs					
hs-cTnI	No AMI (n=1156)			AMI (n=241)		
	Rule-out	Observe	Rule-in	Rule-out	Observe	Rule-in
Rule-out	152	31	1	0	0	0
Observe	305	581	21	1	61	16
Rule-in	1	24	40	0	18	145
NRI	0.240 (95% CI, 0.190-0.270)			-0.012 (95% CI, -0.061-0.036)		
NRI (dimensionless)	0.227 (95% CI, 0.170-0.285); p value <0.001			IDI	0.075 (95% CI, 0.056-0.094)	
Thresholds	**Sensitivity** (95% CI)		**NPV** (95% CI)	**Specificity** (95% CI)		**PPV** (95% CI)
hs-cTnI 2 ng/L	100% (100-100%)		100% (100-100%)	15.9% (14-18%)		19.9% (17.7-22%)
hs-cTnI 52 ng/L	67.5% (61.3-73.7%)		93.3% (91.9-94.7%)	94.4% (93.1-95.6%)		71.5% (65.4-77%)
cMyC 10 ng/L	99.6% (98.6-100%)		99.8% (99.3-100%)	38.1% (35.3-41%)		25.2% (22.4-28.1%)
cMyC 120 ng/L	66.9% (60.7-72.4%)		93.2% (91.7-94.6%)	94.6% (93.3-95.9%)		72.1% (66.2-78.3%)

Legend: NRI = Net Reclassification Improvement; IDI = Integrated Discrimination Improvement; CI = Confidence Interval; NPV = Negative Predictive Value; PPV = Positive Predictive Value; AMI = Acute Myocardial Infarction, based on the adjudicated gold-standard diagnosis

Table S13. Specificity of biomarkers at presentation for adjudicated diagnosis of Acute Myocardial Infarction at the 99th centile

	cMyC at 87 ng/L	***hs-cTnI at 26 ng/L***	***hs-cTnT at 14 ng/L***
Sensitivity	69.6% (95% CI, 64.9-74.2%)	70.6% (95% CI, 65.6-75.5%)	91% (95% CI, 87.8-94.1%)
Specificity	93% (95% CI, 91.7-94.3%)	92.3% (95% CI, 90.8-93.5%)	76.4% (95% CI, 74.3-78.6%)
NPV	93.6% (95% CI, 92.3-94.7%)	93.8% (95% CI, 92.5-94.9%)	97.6% (95% CI, 96.7-98.4%)
PPV	67.7% (95% CI, 62.9-72.4%)	65.4% (95% CI, 60.2-70.6%)	44.4% (95% CI, 40.7-48.2%)

Table S14. Prognosis – Harrell's C and Somers' D statistics

n=1876	**cMyC**	**hs-cTnT**	**p value***	**est.cov**
FU AMI				
Harrell's C Statistic	0.725	0.706	0.251	0.000
Somers' D ± SD	0.450 ±0.045	0.411 ±0.048		
FU death				
Harrell's C Statistic	0.765	0.782	0.142	0.000
Somers' D ± SD	0.530 ±0.034	0.564 ±0.031		
FU composite EP				
Harrell's C Statistic	0.745	0.749	0.667	0.000
Somers' D ± SD	0.489 ±0.029	0.498 ±0.029		
n=1857	**cMyC**	**hs-cTnI**	**p value**	**est.cov**
FU AMI				
Harrell's C Statistic	0.724	0.714	0.577	0.000
Somers' D ± SD	0.447 ±0.047	0.429 ±0.047		
FU death				
Harrell's C Statistic	0.767	0.732	0.001	0.000
Somers' D ± SD	0.535 ±0.034	0.464 ±0.036		
FU composite EP				
Harrell's C Statistic	0.746	0.722	0.008	0.000
Somers' D ± SD	0.492 ±0.029	0.443 ±0.030		
n=1774	**cMyC**	**s-cTnI**	**p value**	**est.cov**
FU AMI				
Harrell's C Statistic	0.719	0.504	<0.001	0.000
Somers' D ± SD	0.438 ±0.047	0.007 ±0.002		
FU death				
Harrell's C Statistic	0.763	0.507	<0.001	0.000
Somers' D ± SD	0.527 ±0.035	0.014 ±0.011		
FU composite EP				
Harrell's C Statistic	0.741	0.503	<0.001	0.000
Somers' D ± SD	0.483 ±0.030	0.007 ±0.008		

Legend: FU = Follow-up event, AMI = Acute Myocardial Infarction (based on the adjudicated gold-standard diagnosis), composite EP = endpoint combining death and AMI during FU (excluding index event), Somers' D quoted ± SD = Standard error of Somers' D, est.cov = estimated covariance between two C indices; *p value for direct comparison between biomarkers

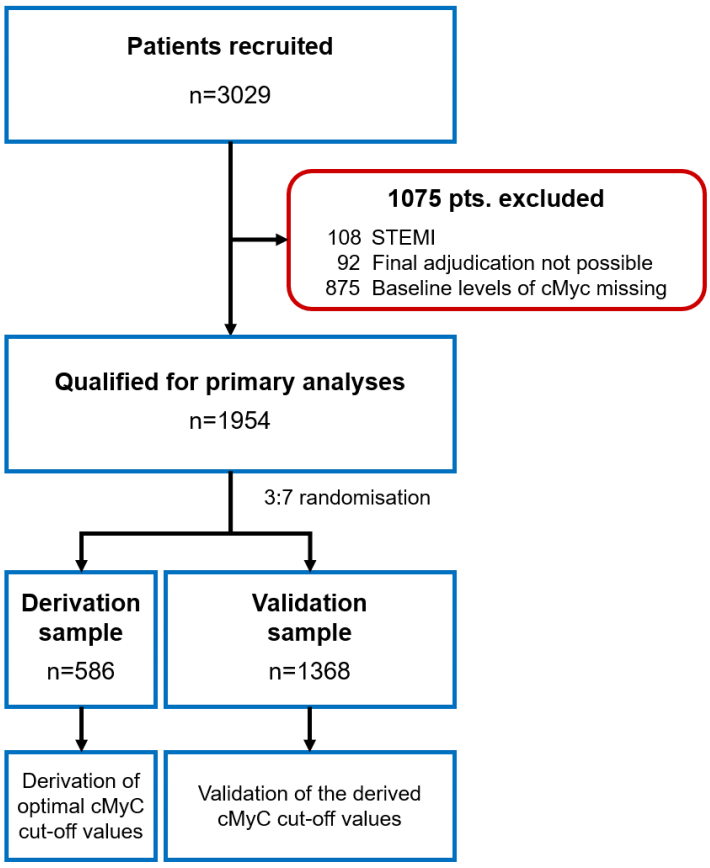


Figure S1

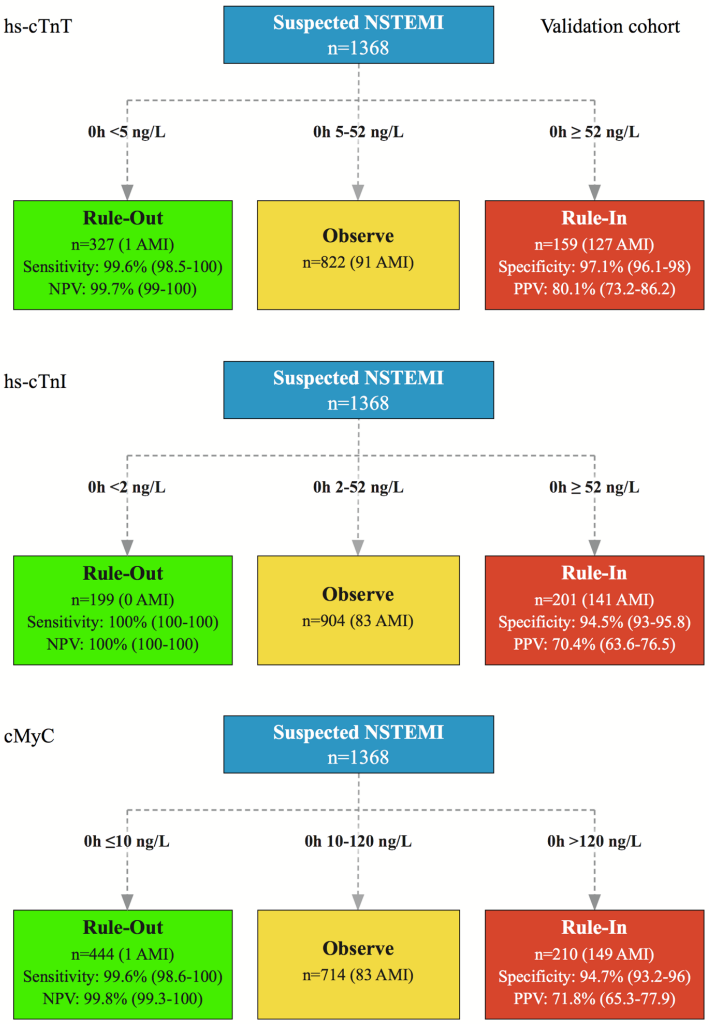


Figure S2

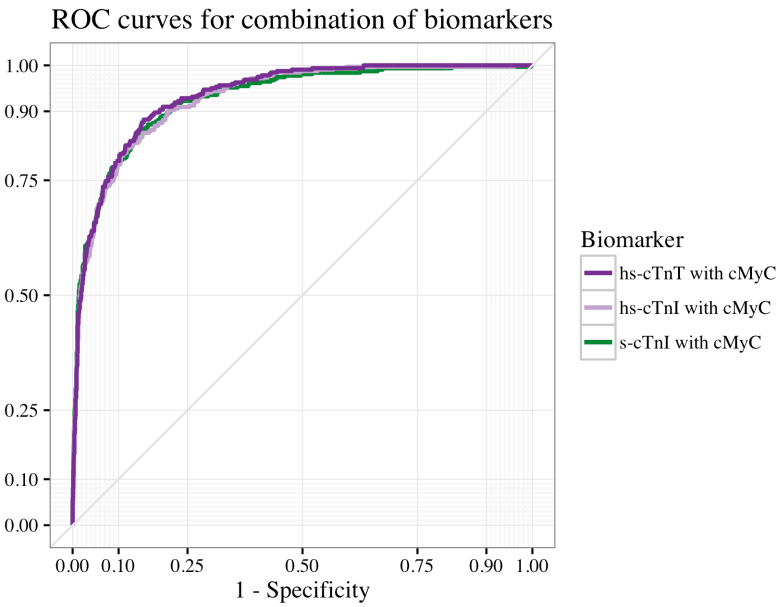
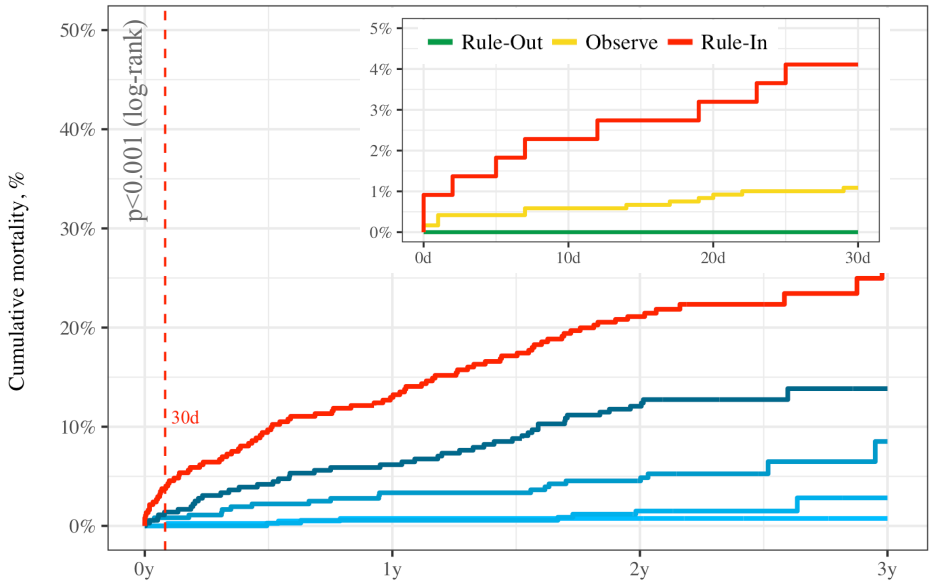


Figure S3

Survival curve for hs-cTnT - 3 year follow-up



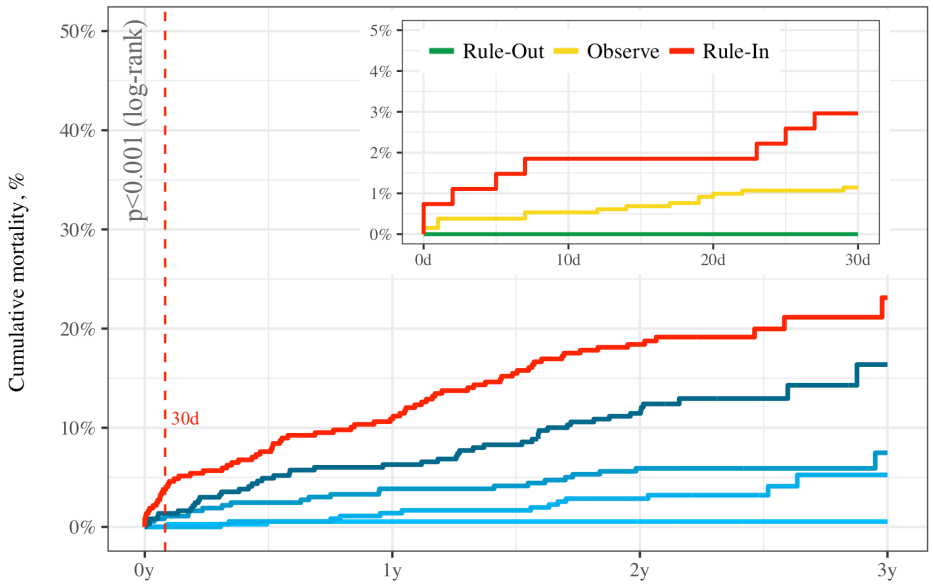
Number at risk at 30d

	10d	20d	30d
Rule-Out	457	457	456
Observe	1194	1187	1183
Rule-In	217	214	212

Number at risk at 3 years

	1y	2y	3y
1st quintile	407	391	351
2nd quintile	367	352	300
3rd quintile	364	343	296
4th quintile	359	329	275
5th quintile	371	318	259

Survival curve for hs-cTnI - 3 year follow-up



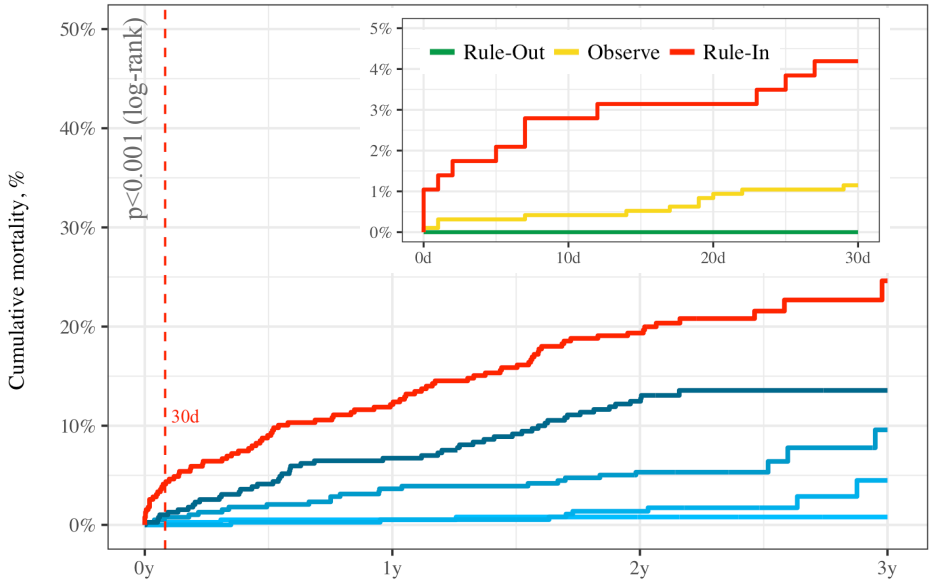
Number at risk at 30d

	10d	20d	30d
Rule-Out	271	271	271
Observe	1309	1303	1296
Rule-In	269	265	265

Number at risk at 3 years

	1y	2y	3y
1st quintile	379	367	315
2nd quintile	366	354	315
3rd quintile	368	344	299
4th quintile	368	340	288
5th quintile	368	319	261

Survival curve for cMyC - 3 year follow-up



Number at risk at 30d

	10d	20d	30d
Rule-Out	706	705	704
Observe	956	953	948
Rule-In	284	278	277

Number at risk at 3 years

	1y	2y	3y
1st quintile	390	377	327
2nd quintile	388	374	324
3rd quintile	390	365	320
4th quintile	391	356	297
5th quintile	387	335	283

Figure S4A-C 1st quintile 2nd quintile 3rd quintile 4th quintile 5th quintile

Supplemental figure legends:

Figure S1: Flowchart outlining recruitment numbers and exclusions from test cohort

Figure S2: Flow of participants, depending on each biomarker used, according to ESC guideline[6] for hs-cTnT and hs-cTnI, and theoretical model for the novel biomarker cMyC; AMI = Acute Myocardial Infarction, based on the adjudicated gold-standard diagnosis

Figure S3: ROC curves describing the diagnostic performance of the combination of cMyC with hs-cTnT (dark purple line; AUC 0.935*), hs-cTnI (light purple line; AUC 0.929) and s-cTnI (green line; AUC 0.928*); *p<0.05

Figure S4: Cumulative incidence of death in all patients based on biomarker value at presentation: all-comers underwent follow-up for up to 3 years. Survival curves are plotted for hs-cTnT, hs-cTnI and cMyC based on quintiles for a three year follow-up, and separated in risk groups 'Rule-Out', 'Observe' and 'Rule-In'[6] at 30 day follow-up. Amongst quintiles, the HR for hs-cTnT at three year follow-up was 2.3 (95% CI, 0.6-9.0) in the second quintile, 7.7 (95% CI, 2.3-25.8) in the third, 17.7 (95% CI, 5.5-57.1) in the fourth and 33.6 (95% CI, 10.6-106.3) in the fifth quintile. The HR for hs-cTnI was 6.6 (95% CI, 1.5-29.2), 11.3 (95% CI, 2.7-48.3), 25.1 (95% CI, 6.1-103.3) and 39.7 (95% CI, 9.7-161.8), respectively. The HR for cMyC was 2.6 (95% CI, 0.7-10.0), 7.8 (95% CI, 2.3-25.9), 17.2 (95% CI, 5.4-55.0) and 29.4 (95% CI, 9.3-93.2).

The quintiles comprise of the following tiers:

hs-cTnT (figure S3a): [0.0, 4.1) [4.1, 7.1) [7.1, 12.1) [12.1, 27.5) [27.5, 1750.0]

hs-cTnI (figure S3b): [0.2, 2.3) [2.3, 3.6) [3.6, 6.8) [6.8, 22.9) [22.9, 25351.6]

cMyC (figure S3c): [1.27, 6.92) [6.92, 12.24) [12.24, 24.19) [24.19, 71.71) [71.71, 4369.03]

Additional HTML-document:

Interactive_ROC.html: This represents an interactive document allowing the reader to assess different cut-offs of the respective biomarkers in isolation as well as in comparison to cMyC. This can be further adjusted to match publishing guidelines if the journal wishes to include in the web supplement.

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